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AND

THE PARASITISM OF NEOCOSMOSPORA VASINFECTA SMITH

BY

E. J. BUTLER, M.B., F.L.S.,
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CHAPTER I.

THE PIGEON-PEA NECTRIA.

IN 1906 the writer (1) [1] described diseases of pigeon-pea (Cajanus indicus) and black pepper in India, with all the characters of the large group of wilt diseases induced by parasites of the genus Fusarium, which have recently attracted the attention of mycologists in many parts of the world.

The symptoms on pigeon-pea were such as would arise from a total or partial stoppage of the water-supply from the roots to the aerial parts of the plant. The result was a "wilting" or withering, more or less sudden according to the degree to which the root system was involved, and the intensity of transpiration at the time. The xylem vessels of the root were in all cases found to be plugged by masses of hyphæ, offshoots from which extended into the parenchyma of the central cylinder and into the cortex. On short simple branches within the larger vessels small elliptical spores were found budded off apically; pure cultures obtained from these proved them to belong to the form-genus Cephalosporium. Within the vessels were also sometimes found special durable thick-walled cells (chlamydospores) attached to the hyphæ in various ways and capable of germinating on being sown in water.

1 These numbers refer to the bibliography at the end of the present paper.

Besides these two spore-forms found attached to the hyphæ within the plant tissues, two others were found on the surface of the bark over blackened areas which occur on diseased plants at the base of the stem. One of these was a Nectria, characterised by bright red perithecia within which were numerous cylindrical asci, each with eight colourless, bicellular spores. The other, which was always present in great quantity on the bark, was a Fusarium, a genus the members of which are in several cases known to be merely the lower stages of species of Nectria and allied fungi.

Pure cultures made from the Nectria ascospores gave rise in turn to Cephalosporium, Fusarium and chlamydospores indistinguishable from those found as described above on the diseased plant. The Nectria form itself was not again obtained in these cultures, though some of them were maintained for more than two years on a large variety of media. It was, however, once obtained in culture from the similar fungus found on black pepper.

Infection experiments with pure cultures of the fungus were carried out. As mentioned on page 28 of the previous paper, the results were irregular, the inoculated plants taking the disease in some of the experiments and not in others.

As the details of these were not given, the results of a few may be summarised here.

Experiments with Nectria and Cephalosporium from wilted Pigeon-pea.

A preliminary experiment showed that infective material occurred on the surface of diseased roots and of the base of the stem, a washing from these parts after they had lain for two days in a moist chamber, proving fatal to healthy plants.

Series I.

The culture used was a nutrient gelatine subculture from a plating from the surface of the bark of a wilted plant, overlying a blackened area in the wood where the tissues were full of hyphæ,

and chiefly consisting of Fusarium spores. The subculture was salmon-pink in colour, as the Fusarium beds on the bark of the wilted plants are. It was determined microscopically to contain both unicellular conidia formed at the tip of short lateral branches and elongated curved several-celled spores, and to agree entirely with the Cephalosporium and Fusarium stages of the Nectria. It bore no perithecia either then or in subsequent subcultures.

The plants were grown in 24 flower pots, seeds, soil, and pots being unsterilised.

The inoculation was made by sprinkling the culture, broken up in distilled water, over the seeds and soil, after sowing but before covering with soil, 12 pots being thus treated, while 12 were left uninoculated to serve as controls.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
32 plants in 12 pots.	Inoculated with Fusarium and Cephalosporium spores.	Sown and inoculated. 22-11-04.	1 plant wilted on 23-1-05. 3 plants wilted on 25-1-05. 2 plants wilted on 29-1-05. 1 plant wilted on 30-1-05.	All the remaining plants were killed by frost on the night of 30-1-05.
27 plants in 12 pots.	Not inoculated (control).	Sown 22-11-04.	No deaths up to 30-1-05.	

This experiment showed that pure cultures of a fungus present on the bark of wilted pigeon-pea plants and agreeing with the Cephalosporium and Fusarium stages of the Nectria found in the same locality are capable of producing wilt. Examination of the roots of three of the wilted seedlings showed them to be infested with hyphæ in the manner above described, and copious *Cephalosporium* spores were developed on the surface of the roots when they were placed in a moist chamber for a few days.

On February 25th the twelve pots that had been inoculated were resown with pigeon-pea. On April 19th two of this second batch of seedlings wilted in one pot. The roots of these were examined and showed clear signs of fungus-attack. Many of the lateral roots

were marked by shrunken blackened patches, in some cases near the tap root, in others some distance away. Most of the fine rootlets arising from these had been killed, and the appearances suggested that the disease had originated in the latter and extended backwards to the larger roots. The tap root itself was unaffected. The parasite, therefore, remains in the soil and is capable of infecting successive crops of pigeon-pea, a fact which is besides only too evident from field experience of the disease. Observation of the remaining seedlings ceased soon after April 19th owing to my absence from head-quarters.

Series II.

The culture used was the 5th successive subculture (on pigeon-pea slab) from an ascospore of the Nectria already mentioned. It contained Cephalosporium and Fusarium spores, but neither it nor the four previous subcultures had given rise to perithecia.

The plants were grown in two pots in the same way as in Series I and sowing was done with germinated seeds.

The inoculation was made after the seedlings were up by scraping away the soil down to a lateral root and applying the culture to this.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
4 plants in 1 pot.	Inoculated with a subculture from Nectria.	Sown 25-2-05. Inoculated 8-3-05.	No deaths up to July 05.	On April 10th made the number of plants up to 6 with germinated seedlings.
6 plants in 1 pot.	Not inoculated (control).	Sown 25-2-05.	,, ,, ,,	

Pure cultures originating from Nectria ascospores, therefore, failed to produce the disease.

Series III.

The culture used was the same as that in Series II.

The plants were grown in 8 pots in the same way as in the previous experiment.

The inoculation was made by sprinkling the soil with the culture broken up in distilled water.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
7 plants in 4 pots.	Inoculated with a sub-culture from Nectria.	Sown 2-3-05. Inoculated 8-3-05.	No deaths up to July 05.	On April 10th made the number of plants up to 6 in each pot with germinated seedlings.
10 plants in 4 pots.	Not inoculated (control).	Sown 2-3-05.	,, ,, ,,	,, ,, ,, ,,

As in the previous experiment cultures from Nectria ascospores failed to produce the disease.

Series IV.

The culture was obtained as in Series I, the colony taken from the plate being one of a large number bearing Cephalosporium spores.

The plants formed part of two long rows growing in a field plot and about a month old.

The inoculation was made as in Series II above.

No. of plants.	Treatment.	Date of inoculation.	Result.
100	Inoculated with Cephalosporium originating from the surface of a wilted pigeon-pea root.	Last week of August 03.	8 plants had wilted by 6-10-03 when observations ceased.
Remainder of the rows (several hundred).	Not inoculated (control).		No deaths.

This experiment, so far as it goes, agrees with the results obtained in Series I.

CHAPTER II.

Neocosmospora vasinfecta Smith.

The above experiments, except Series IV, were carried out at Dehra Dun where the Mycological Laboratory of the Indian Department of Agriculture was then situated, and the material used was all obtained in that locality. Series IV was carried out at Poona. As they were evidently unsatisfactory, work was continued at Pusa in 1907-08. The pigeon-pea wilt is common in the vicinity and provided abundant material for investigation.

Early in 1907 perithecia of the well-known fungus *Neocosmospora vasinfecta* were discovered on the roots of wilted plants. As a large proportion of the wilt diseases of plants hitherto investigated has been attributed to forms of this fungus, the discovery was significant, and further search showed that most of the dead roots examined either already bore these perithecia or could be induced to produce them by incubation in a moist atmosphere. The most careful search carried on for two years at Pusa and other localities in the plains of India has been unsuccessful in finding the Nectria met with at Dehra Dun on this host.[1] On the other hand, Neocosmospora vasinfecta has been found on pigeon-pea roots from Dehra Dun and has proved to be widely distributed throughout Northern India.

This fungus was first described by E. F. Smith (2) in the United States, in connection with wilt diseases of cotton, watermelon and cow-pea. The symptoms in all three diseases were

[1] This is not proof that it does not occur in its lower (Cephalosporium and Fusarium) stages. The Erysiphaceæ of the plains are common enough in the conidial stage, but perithecia are rare and in some species are apparently not formed. In the more temperate parts (as in Kashmir) perithecial forms of Erysiphaceæ are common. It is quite possible that the temperate climate of Dehra Dun favours the production of perithecia in the pigeon-pea Nectria, whereas the heat of the plains checks this stage and forces the fungus to reproduce by means of conidia alone.

similar and quite agree with those described above for pigeon-pea wilt. Within the tissues were found small unicellular conidia (microconidial or Cephalosporium stage), and on the surface larger falcate spores with several septa (macroconidial or Fusarium stage). Further, bright red perithecia occurred frequently on the roots of wilted plants and were proved by single-spore culture to give rise to micro-and macroconidia indistinguishable from those found in nature on diseased roots, and finally, in old cultures, to chlamydospores similar to those found accompanying the microconidia on the bark. The perithecia differ from those of Nectria in being found usually on the roots (whereas the pigeon-pea Nectria occurs on the stem, never on the roots) and in containing characteristic unicellular spores with a thick wrinkled brown wall. For this hitherto undescribed fungus Smith established the genus Neocosmospora, with one species N. vasinfecta (Syn. Fusarium vasinfectum, Atk.) on cotton and varieties tracheiphila and nivea on cow-pea and water-melon respectively.

The biology of the fungus was very fully investigated by Smith, particularly its behaviour as a pigment-producer, in which it shows certain definite characters. His experiments regarding its powers as a parasite led to less definite results, or rather showed that there were points in its life as a parasite that required elucidation. The chief of these were the following.

Though the fungus on cotton and cow-pea resembled that on melons in cultural characters and anatomical details and the diseases on all three were similar, he entirely failed to produce wilt in the first two cases by inoculating healthy plants with cultures of the fungus associated with the disease. In the water-melon on the other hand disease was easily induced by pure cultures of the fungus obtained from melon roots. All attempts at cross inoculations, using the fungus obtained from one host to inoculate the other two, failed. Hence, as stated on p. 46 of his paper, the parasitism of the variety on water-melon was fully established, while that of those on cotton and cow-pea was "not proved."

The virulent strain of the water-melon fungus was isolated from an internal microconidium and maintained its virulence as long as the work extended, through several years. No perithecia ever appeared in these cultures or in any made from external or internal conidia taken from cotton or water-melon. The genetic connection of the conidial forms on water-melon was proved, but not their connection to the perithecia. No inoculations were made with cultures derived from ascospores from the perithecia of the water-melon fungus. Hence, while it was proved that the water-melon wilt is due to a fungus possessing microconidial (Cephalosporium) and macroconidial (Fusarium) stages, it was not proved that this fungus is Neocosmospora vasinfecta ; the only evidence being that Neocosmospora possesses exactly similar stages.

Orton (3) in a paper published the year following Smith's, mentions that the cotton wilt had been subsequently produced in healthy cotton plants by inoculating the soil in which they grew with pure cultures of conidial stages of Neocosmospora vasinfecta. It is not, however, stated that these cultures were derived from ascospores ; in other words, it is not proved (so far as the published observations go) that the fungus used really belonged to Neocosmospora vasinfecta.

More recently Reed (4) has endeavoured to throw doubt on the parasitism of Neocosmospora, in the case of a wilt disease of ginseng (Aralia quinquefolia). The disease agrees with that described by Smith and the fungus present resembles the conidial stages of Neocosmospora, to which Reed refers it while holding that it always follows in the path of other fungi, and is, therefore, only a weak parasite. As, however, perithecia were not observed, there is no evidence at all that Reed's work was done with Neocosmospora. When this was pointed out by Smith, Reed subsequently admitted that the two fungi may not prove to be identical.[1]

Outside of the United States wilt diseases of various plants have been attributed to Neocosmospora vasinfecta in several

[1] See discussion in Science, New Series, Vol. XXVI, 1907, under heading, "The parasitism of Neocosmospora—inference versus fact" by E. F. Smith, p. 347, and "The parasitism of Neocosmospora" by H. S. Reed, p. 441.

instances. Delacroix (5) referred to it the Egyptian cotton wilt, and in the same paper suggested that the cause of a disease of carnations, previously investigated by him (17), was identical: Jaczewski (6) that of sesamum in Turkestan where he has also recorded it on cotton and Hibiscus (7) : Malkoff that of cotton in Sadovo (8) : Zimmermann that of cotton in German East Africa (9) : and van Hall that of the same host in the Dutch West Indies (10). In these cases, except Zimmerman's, perithecia were not found and the identification was made from conidial stages alone.

The absence of perithecia has led several observers to exercise greater caution in the identification of the parasites. Thus the "St. Johannis" disease of peas in Holland was attributed by van Hall (11) to "Fusarium vasinfectum Atk. var. Pisi," a terminology which while implying specific identity with the conidial stages of Smith's fungus, leaves the genetic connection with Neocosmospora open. Similarly Farneti (12) and Montmartini (13) used the terms Fusarium niveum Sm. and Fusarium vasinfectum Atk. for wilt diseases of cucurbitaceous plants and of Capsicum respectively in Italy.

In the important paper published by Schikorra (14) the name Fusarium vasinfectum Atk. var. Pisi van Hall is retained for the parasite of the "St. Johannis" diseases of peas, but the author is careful to point out (p. 173), that further investigation is necessary to decide how far the various Fusaria found within the vascular bundles of cultivated plants belong to one or to several different species.

So far as I have been able to ascertain, Neocosmospora vasinfecta Sm. has only been found in its perithecial stage, outside of the United States, by Zimmermann in German East Africa until its discovery in India in 1907.

The resemblance between its conidial stages and those of the Nectria found on wilted pigeon-pea in Dehra Dun was at once evident. Figs. 3, 4 and 5, plate II, of the writer's previous paper may be compared with fig. 6, plate V, fig. 9, plate III, and fig. 7,

plate II, of Smith's paper on this point. Microscopical preparations taken from cultures on nutrient agar, showed no definite character by which the conidial stages of the two fungi could be differentiated.

As the successful infection experiments carried out at Dehra Dun were made with conidia obtained from the roots of pigeon-pea and not directly from Nectria ascospores, it at once became evident that these conidia might have belonged to either of two distinct fungi, Nectria or Neocosmospora, and that in this might lie the explanation of the irregular results obtained. It appeared possible that the successful experiments might have been made with conidia belonging to Neocosmospora, but erroneously taken for those of Nectria. This became the more probable when it was found that Neocosmospora occurs not only at Dehra Dun, but throughout a large area of Northern India, where pigeon-pea wilt is common, and also that on almost every diseased root examined, Neocosmospora perithecia should be induced to form by appropriate treatment. Work was therefore continued with the latter fungus.

Experiments with Neocosmospora vasinfecta Smith on Pigeon-pea.

To test the parasitism of Neocosmospora a series of infection experiments was carried out with pure cultures of the fungus. Only ascosporal cultures or those known to have originated from perithecia were used, since it was evidently useless to work with conidial forms, which there was no means of referring to their proper genera.

Series V.

The cultures used were obtained as follows :—

Some young perithecia of Neocosmospora were washed carefully in several changes of distilled water, and were sown separately in decoction of pigeon-pea. They sprouted freely from the perithecial cells (as most Hypocreaceous fungi will) and produced quantities of Cephalosporium spores.

A plating from one of these sprouting perithecia was made on April 7th, 1907, and several of the resulting colonies bore perithecia in turn. From one of these perithecial colonies, well isolated, a trace of aerial mycelium bearing Cephalosporium spores was again plated and good isolated colonies originating from a single spore were obtained. Subcultures were made from one of these colonies which was marked and showed perithecia a few days after the subcultures were obtained. The media were boiled pigeon-pea slabs, carrot, onion, boiled rice and plantain.

The plants were grown in pots, the earth in which was sterilised by intermittent steaming on three days in the Koch. The soil was damped and packed loosely into perforated kerosine oil tins. Under these conditions the interior of the mass reached about 87° C. after two hours' steaming, and was kept at this temperature for about three hours each day. Higher temperatures were not obtained with the ordinary Koch steriliser, but the above may be taken as sufficient to kill all life, but the most heat resisting bacteria in soil. Subsequently (in 1908) a Hearson's thermostatic dry steam steriliser was used and a temperature of 100° C. throughout was easily reached with this. The soil was loaded into twelve unsterilised pots, in each of which about twenty-five unsterilised seeds were sown. Germination was good.

The inoculation was made by sprinkling the soil with the culture broken up in distilled water just after the plants were up.

No. of pot.	Treatment.	Date of inoculation.	Result.	Remarks.
I	Inoculated with culture on pigeon-pea slab.	11-5-07.	No deaths.	
II	,, ,, ,, ,,	,,	,, ,,	
III	,, ,, ,, carrot	,,	,, ,,	
IV	,, ,, ,, onion	,,		
V	,, ,, ,, rice	,,	Wilt appeared.	Two plants wilted on July 3rd. All were dead but one on August 14th.
VI	,, ,, plantain	20-5-07.	No deaths.	
VII	Not inoculated (control)	...	,, ,,	
VIII	,,	...	,, ,,	
IX	,,	...	,, ,,	
X	,,	...	,, ,,	
XI	,,	...	,, ,,	
XII	,,	...	,, ,,	

Wilt appeared in pot No. V only. The subsequent history of the contents of this pot will be given below under Series XIV (p. 36). The plants from the remaining eleven pots were transplanted into my garden on August 14th, having become too large for their pots. On September 8th those from pots II and III were attacked at the collar by a swarm of small red ants, and several were killed. One of these was pulled out on October 9th, and bore numerous perithecia of Neocosmospora on the roots. The rest remained healthy up to July 1908, and will be referred to again under Series XVII.

Series VI.

The cultures used were sub-cultures on nutrient agar, rice and onion from the rice tube used in the successful inoculation in Series V (pot V).

The plants were those growing in pots I, III, IV and VI of Series V. They were transplanted into my garden soon after inoculating.

The inoculation was made in the case of pot I by scraping away the soil from the base of the stem until lateral roots were exposed, scraping off the bark from these and placing a piece of the culture watered with distilled water on the wound, and in the case of the other three by simply watering the soil with the culture broken up in distilled water.

No. of pot.	Treatment.	Date of inoculation.	Result.
I	Inoculated at a wound with culture on rice.	27-7-07.	No deaths up to July 1908.
III	Inoculated by watering with rice, onion and agar cultures.	12-8-07.	On September 8th several plants killed by ants, rest remained healthy up to July 1908.
IV	,, ,, ,,	,,	No deaths up to July 1908.
VI	,, ,, ,,	,,	,, ,, ,,

Though the plants in pot V of Series V, inoculated from a rice culture of Neocosmospora, contracted wilt, subcultures from the

same culture failed in the inoculations of this series. Hence the infection could not have come from the culture but from another source, probably either from the unsterilised seed or pot-walls or from the air.

Series VII.

The cultures were subcultures on nutrient agar, rice and onion from the rice tube used in the successful inoculation in Series V (pot V).

The plants were fifty consecutive plants in a row in the centre of a field plot on the Pusa Farm, and were about 18 inches high.

The inoculations were made as in Pot I of Series VI.

No. of plants.	Treatment.	Date of inoculation.	Result.	Remarks.
50	Inoculated at a wound with mixed agar, rice, and onion cultures.	11-8-07.	4 deaths.	Perithecia of Neocosmospora found on 2 dead plants.
Remainder of a plot of about $\frac{1}{10}$ acre.	Not inoculated (control).	...	7 deaths.	Perithecia of Neocosmospora found on one dead plant.

The proportion of deaths in the inoculated row was not sufficient, when compared with the deaths in the remainder of the plot, to afford proof that the former had died as a result of inoculation.

Series VIII.

The culture used was obtained by plating ascospores from a perithecium on pigeon-pea root, the ascospores being tested and found to germinate freely in water. In one plate ten well-isolated colonies of Cephalosporium appeared, and all bore perithecia on the 8th day. From one a subculture was taken on the 5th day, and this subculture bore perithecia after four days. From it, when two days old, three subcultures were made on glucose agar. These were used for the inoculation when eleven days old and with numerous perithecia.

The plants were grown in a small plot 8 by 4 feet, in my garden, a similar plot being sown as control. The plots were separated by a trench about a foot in depth. The seeds were sterilised by steeping in ·1 per cent. formaldehyde for two hours.

The inoculations were made by sprinkling the seeds and soil after sowing, but before covering, with the cultures broken up in distilled water.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.
Contents of a plot, 8 by 4 feet.	Inoculated by sprinkling with glucose agar cultures.	Sown and inoculated 18-7-08.	Seven plants died between the 10th and 25th August 1908. No more of the several hundred plants died up to the end of the year.
,, ,, ,,	Not inoculated (control).	Sown 1-7-08.	No deaths up to October 26th, 1908.

I am unable to explain the seven deaths in the inoculated plot. Not a single death occurred subsequently to August 25th, and this is almost conclusive evidence that the deaths were not due to true wilt. I have never seen a case of the latter where sooner or later neighbouring plants have not taken the disease. In a neighbouring plot affected by true wilt (that described under Series XVI below), deaths were numerous and spread was continuously going on up to early in the following year.

Series IX.

The culture used was a subculture originating from the first subculture from the plating in Series VIII.

Subcultures were taken once a month and all gave very numerous perithecia during the four months which elapsed between Series VIII and IX.

The plants were grown in pots which were immersed in ·001 per cent. corrosive sublimate solution for about five minutes before filling with soil. The soil was sterilised by intermittent steaming

for three days, the internal mass being maintained at 100° C. for about two hours each day. The seeds were sterilised by steeping in ·1 per cent. formaldehyde for two hours.

The inoculations were made by sprinkling the seeds after sowing, but before covering, with the culture broken up in distilled water.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.
26 plants in 4 pots.	Inoculated with a culture on glucose agar.	Sown and inoculated 1-11 08.	No deaths up to 5-3-09.
17 plants in 2 pots.	Not inoculated (control).	Sown ,, ,, ,,	,, ,, ,,

No difference in growth could be detected between the inoculated and control pots. All the plants appeared equally healthy.

Series X.

Attempts were made to infect pigeon-pea with pure cultures derived from the ascospores of Neocosmospora obtained from the roots of other host plants.[1] Cotton, indigo, and gram were selected.

The culture used in the case of the cotton Neocosmospora originated from a plating of ascospores from a perithecium on the root of a wilted cotton plant from Sind. The subcultures were taken from an isolated Cephalosporium colony and bore perithecia in about a week. The series was maintained by regular subculturing every few weeks, and was fourteen months removed from the parent plating at the time of the inoculations below. Every subculture of the series bore quantities of perithecia. That used in the inoculation was about a fortnight old.

In the case of the indigo Neocosmospora some perithecia from a wilted indigo (Indigofera sumatrana) plant were well washed

[1] See below, p. 18 *et seq.*

in distilled water and crushed to obtain ascospores. These were plated. A good growth occurred in the trace of agar left on the walls of one of the tubes used for plating and was seen under the microscope to arise from a group of three or four ascospores. From this an agar tube was inoculated and the subculture thus obtained showed perithecia on the fifth day. Seven days later three subcultures were made from the first and gave microscopically pure growths, which bore numerous perithecia when examined after twelve days. Two of these tubes were used for the inoculations, the cultures being twelve days old.

In the case of the gram Neocosmospora a plating was made of mature ascospores from a root of a wilted gram plant obtained from Bannu, North-West Frontier Province, and preserved in the laboratory for a year. Several good isolated Cephalosporium colonies resulted and a subculture from one, grown on agar, proved microscopically pure and bore numerous perithecia after five days. Subcultures were maintained regularly, all of which bore perithecia in large quantity. That used in the inoculations was five months removed from the plating and was about a week old.

The plants were grown in pots, the sterilisation of pots, soil and seeds being the same as in Series IX, except in the case of the indigo fungus where the pots were not sterilised.

The inoculations were made as in Series IX, except in the case of the indigo fungus where the seeds were sown in pots after pulling out indigo plants which had been previously watered with the cultures broken up in distilled water. In this and in subsequent tables the control experiment, when one was employed, is always given immediately below the experiment to which it relates.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
16 plants in 2 pots.	Inoculated with cotton Neocosmospora.	Sown and inoculated on 1-11-08.	No deaths up to 5-3-09.	

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
17 plants in 2 pots.	Not inoculated (control).	Sown on 1-11-08.	No deaths up to 5-3-09.	
About 50 plants in 3 pots.	Sown in soil previously inoculated with indigo Neocosmospora.	Sown on 28-3-08.	No deaths up to 26-10-08.	Transplanted to garden soil on 1-7-08, the plants having become too big for the pots.
8 plants in 2 pots.	Inoculated with gram Neocosmospora.	Sown and inoculated 1-11-08.	No deaths up to 5-3-09.	In another experiment pigeon-pea was sown in 4 pots previously inoculated with gram Neocosmospora. 45 plants grew and no deaths occurred in 4½ months.
17 plants in 2 pots.	Not inoculated (control).	Sown on 1-11-08.	,, ,, ,,	

Attempts to produce pigeon-pea wilt by inoculating with ascosporal cultures of Neocosmospora obtained from the roots of wilted cotton, indigo and gram failed.

Summary of experiments with Neocosmospora vasinfecta Smith on Pigeon-pea.

The above experiments (Series V to X), in which attempts were made to induce pigeon-pea wilt by inoculating with Neocosmospora, may now be considered as a whole. Apparently successful results were obtained in Series V (one pot out of six inoculated), Series VII (four plants out of fifty inoculated), and Series VIII (seven plants out of several hundred inoculated).

In each of these cases the experiment was not carried out under conditions which precluded the entry of foreign spores. In Series V the pots and seed were not sterilised, and it is proved by Series VI that the infective material did not arise from the tube with which the inoculation was made. Further work, the details of which will be found under Series XIV on p. 36 below, showed that the soil of this pot contained eventually another fungus which there is abundant evidence to prove is the real cause of pigeon-pea wilt. Series VII and VIII were field experiments, in which, of course, the soil was not sterilised. The four deaths out of the fifty inoculated

plants in the former were accompanied by seven deaths in the non-inoculated portion of the plot. No significance therefore attaches to them, except as showing that the infective material is not uncommon in local soils. In Series VIII there is good reason to suppose that the deaths were not due to wilt.

On the other hand, in Series IX, where all the material used was rigidly sterilised, no deaths occurred, nor did any in Series VI (if we exclude the few plants killed by ants), though the seeds and pots were not sterilised and the plants were transplanted into open garden soil soon after inoculation. In five out of the six pots inoculated in Series V, no death occurred, nor did any deaths occur in Series X, in which pigeon-pea was inoculated with Neocosmospora from cotton, indigo and gram.

Even though taken as negative evidence, the results so far given are sufficient to justify the conclusion that the Neocosmospora found on the roots of wilted pigeon-pea, cotton, indigo and gram plants in India is not the cause of the disease in the first of these crops ; the positive results obtained with another fungus detailed in Series XIV to XVII below leave no room for doubt on this point.

Experiments with Neocosmospora vasinfecta Smith on Cotton, Indigo and Gram.

The experiments with Neocosmospora on pigeon-pea must give rise to doubts regarding the generally believed parasitism of this fungus. The disease of this crop in India is a typical wilt, closely resembling those of cotton and other plants in the United States, where the cause is definitely stated to be this fungus, and of peas in Europe, where the same is suggested by the use of a name (Fusarium vasinfectum) given by Smith as a synonym for Neocosmospora vasinfecta. It is unlikely that what is a virulent parasite on widely different hosts in other countries should be a harmless saprophyte on another host suffering from a similar disease in India. The fact that, so far as published statements show, all successful inoculations obtained in other countries have been made with the

Fusarium and Cephalosporium stages of the parasite strengthens this doubt. It has already been shown above that exactly similar stages are possessed by a fungus of another genus, the Nectria found on wilted pigeon-pea plants at Dehra Dun. It is indeed well known that it is scarcely possible to distinguish by the microscope alone, many of the members of the form-genus Fusarium from one another, and the same applies to an even greater degree to the form-genus Cephalosporium.[1]

Both these form-genera are known to be in many cases lower stages of various genera of the Hypocreaceæ, and there are few groups amongst the fungi where it is more difficult to determine the genus by observation of conidial stages alone. In the present state of our knowledge it is not possible to refer a Fusarium or a Cephalosporium to its ascomycetal genus without actually deriving it in culture from the perithecial stage. This does not appear to have been done in the cultures with which successful inoculations of wilts have been carried out, and on this account the question of the parasitism of Neocosmospora cannot be regarded as settled.

Wilt diseases, associated with the presence of Neocosmospora on the roots, have been found in the following crops in India :— Pigeon-pea, cotton, indigo, gram (Cicer arietinum), sunn-hemp (Crotalaria juncea) and cow-pea (Vigna Catiang). Of these, cotton, indigo, and gram were selected for a further test of the parasitism of the fungus. In planning the experiments it was decided to try inoculations with the fungus from each of the three crops, and to add that from pigeon-pea in each series.

Cotton Wilt.

Cotton wilt has been reported from Sind, Gujarat, the Central Provinces and the Punjab in India. In the two latter areas it has caused considerable loss in the past few years. Perithecia of

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1 *c. f.* for Fusarium the synonyms given on page 50 of Smith and Swingle's paper on the "Dry rot" of Potatoes (Bibliography (15)), and the remarks by Schikorra ((14), p. 176) and for Cephalosporium Brefeld's Untersuchungen, Vol. X. Plates IV and V amongst others.

Neocosmospora were found on the roots of wilted plants from the Central Provinces and Sind.

In the Central Provinces the disease is said to have been known for the past 50 years. It appears to have become more severe recently, possibly as suggested by Evans (16) because, owing to the recent cotton boom, the crop has been grown without proper attention to rotation. In parts of the Saoner tract it was estimated that 5 per cent. of the crop was killed in places in 1907. In Akola the percentage of deaths was 30 on an area of 80 acres severely affected in 1908. It was noticed that varieties of Gossypium neglectum and G. indicum are chiefly attacked, G. hirsutum, as represented by the kind known as *buri*, an upland Georgian type, being immune.

In Akola the wilting began soon after germination and went on progressing from the originally attacked plants all through the season. Details of the attack on the Nagpur Experimental Farm in 1907 are given in Evans' note just mentioned.

The examination of the specimens sent in from these two localities disclosed a condition entirely agreeing with the published descriptions of the disease in the United States. The Akola specimens were young plants, and externally appeared sound except for a discolouration of the bark at the base of the stem and on the tap root. On removing this the tissues below were found blackened just as in pigeon-pea wilt, and sections showed that many of the xylem vessels were quite choked with hyphæ. On these microconidia, quite agreeing with those figured by Smith, were found. Chlamydospores similar to those formed by many Fusaria were also formed within the vessels and Fusarium beds were common on the bark. There is practically no doubt that the disease is a Fusarium wilt, and it cannot be distinguished from that of the United States and Egypt.

Series XI.

(*a*) *Cotton inoculated with Neocosmospora vasinfecta from cotton.* The culture used was one of the earliest of the series mentioned under Series X.

The plants were grown in pots sterilised by immersion in ·001 per cent. corrosive sublimate for five minutes before loading with soil. The soil was sterilised by intermittent steaming for three days as in Series V. The seeds were steeped in ·1 per cent. formaldehyde for two hours. The plants in this and the following experiments were of the variety known as *bani*, a variety of Gossypium indicum which is susceptible to wilt ; and part of the same lot of seed sown on the Nagpur Farm was badly attacked by the disease.

The inoculations were made by sprinkling the culture, broken up in distilled water, over the seeds and soil after sowing but before covering.

(*b*) *Cotton inoculated with Neocosmospora vasinfecta from gram.*

The cultures were part of the regular series mentioned under Series X above.

The plants were mature plants of *bani* cotton seven months old, pots and soil being unsterilised but the seeds steeped in formaldehyde as above.

The inoculations were made by scraping away the soil down to the roots and placing the culture on these.

(*c*) *Cotton inoculated with Neocosmospora vasinfecta from indigo.*

The cultures were the same as those used in Series X above.

The plants were grown in pots containing soil to which the cultures had been added about six months previously and which had in the interval grown a crop of indigo, being in fact the same pots as those mentioned below in the 3rd column of Series XII.

The seeds were steeped in formaldehyde before sowing as before.

(*d*) *Cotton inoculated with Neocosmospora vasinfecta from pigeon-pea.*

The culture was that used to inoculate pot V of Series V above.

The plants were grown in pots containing soil to which the culture had been added ten months previously and which had borne

pigeon-pea in the interval. The soil was mixed with enough fresh soil to fill five pots.

The seeds were steeped in formaldehyde before sowing as before.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
About 50 plants in 6 pots.	Inoculated with cotton Neocosmospora.	Sown and inoculated 3-4-08.	No deaths up to March, '09.	Transplanted to my garden on 1-7-08.
Do. do.	Not inoculated (control)	Sown ,,	,, ,,	,, ,,
17 plants in 2 pots.	Inoculated with gram Neocosmospora.	Sown 28-3-08. Inoculated 1-11-08.	,, ,,	
About 30 plants in pots.	Sown in soil previously inoculated with indigo Neocosmospora.	Sown 28-3-08.	No deaths up to November, '08.	Transplanted to my garden on 18-6-08.
About 50 plants in 5 pots.	Sown in soil previously inoculated with pigeon-pea Neocosmospora.	,, ,,	,, ,,	

Though the variety of cotton used in the experiments was one which is highly susceptible to wilt, inoculation with cultures derived from ascospores of Neocosmospora vasinfecta, whether from cotton, gram, indigo, or pigeon-pea, failed to produce the disease in a single instance.

Indigo Wilt.

About the middle of 1907 a serious disease of indigo appeared in Behar, the centre of the indigo industry of India. A few years previously the species of indigo known as "Java" (Indigofera arrecta Hochst.) was introduced experimentally into cultivation. Its superiority to the species previously grown, "Sumatra" (Indigofera sumatrana), was so marked that a rapid extension of its cultivation occurred. The coincidence of the introduction of a new species and the appearance of a disease not previously known in the crop, was such as to lead to a strong suspicion that the disease had been introduced with the seed. Enquiry from Java, whence the

seed originated, showed, however, that the disease was not known there. Its field characters were similar in many respects to those of the wilts described above, and perithecia of Neocosmospora vasinfecta were found to be common on the roots of diseased plants. What connection, if any, exists between the introduction of the new species of plant and the outbreak of disease is not clear, but it appears possible that a parasite already present on some other crop found the new species susceptible to attack and through it passed to the species common in the district. The interesting conception of "bridging species" already established for the host plants of the Erysiphaceæ and rusts may be called into account for the appearance of wilt in indigo. Both the species of indigo, Indigofera sumatrana and I. arrecta, were attacked, but the disease was most severe in the latter.

Inoculation experiments were undertaken with Neocosmospora vasinfecta from indigo, cotton, gram and pigeon-pea.

Series XII.

(*a*) *Indigo inoculated with Neocosmospora vasinfecta from indigo.*

The culture used was obtained as described under Series X from the root of a wilted plant of Indigofera sumatrana, and bore numerous perithecia.

The plants were grown in pots, the pots and soil being unsterilised. The seed of the Sumatra variety was untreated in any way, while that of the Java variety was steeped for 20 minutes in strong sulphuric acid to promote germination, since it belongs to the category of "hard seeds" requiring treatment to rupture or soften the seed coat before sowing.

The inoculations were made by sprinkling the seed and soil after sowing but before covering, with the culture broken up in distilled water.

A second series was made, using a culture derived from a wilted plant of Indigofera arrecta.

The culture was obtained by planting ripe ascospores obtained from the root of a plant in a badly diseased field. On the fourteenth day a colony, bearing perithecia, was selected and an agar tube inoculated with a trace of the aerial mycelium. This gave a pure growth which produced perithecia after five days. Subcultures from this were made and used for the inoculations when three days old.

The plants were grown in pots, pots and soil being sterilised as in Series XI (*a*). Half the number of pots were sown with seed derived from plant imported from Java and grown for two generations in India; the seed used having been carefully gathered from plants of the same field as that from which the fungus was obtained, and showing the earliest signs of disease. It was therefore of a susceptible strain. The other half were sown with Indigofera arrecta seed originating from Natal, and which had also shown itself to be susceptible. All the seed was treated with strong sulphuric acid for 20 minutes.

The inoculations were made as before.

(*b*) *Indigo inoculated with Neocosmospora vasinfecta from cotton.*

The culture used was one of the regular series mentioned above, under Series X. It bore numerous perithecia.

The plants were those in one of the control pots of (*a*) above, being Java seed two generations in India, and were nearly three months old.

The inoculations were made by scraping away the soil down to the roots and applying the culture broken up in distilled water.

(*c*) *Indigo inoculated with Neocosmospora vasinfecta from gram.*

The culture used was one of the regular series mentioned above, under Series X, and bore numerous perithecia.

The plants were those in two of the control pots of (*a*) above, being Java seed two generations in India, and were nearly seven months old.

The inoculations were made as in (*b*) above.

(*d*) *Indigo inoculated with Neocosmospora vasinfecta from pigeon-pea.*

The culture used was that used to inoculate pot V of Series V above.

The plants were grown in pots containing soil to which the culture had been added several months previously and which had grown pigeon-pea in the interval. The soil was mixed with enough fresh soil to fill four pots. Two were sown with untreated Sumatra seed and two with Java seed, treated for 15 minutes in strong sulphuric acid.

No. of plants and variety.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
About 50 plants of I. arrecta from Java seed in 4 pots.	Inoculated with Neocosmospora from I. sumatrana.	Sown and inoculated 21-8-07.	No deaths up to July, '08.	All the plants in 1 pot were wounded by cutting down to the cambium below the ground level, 8 weeks after sowing.
Ditto.	Not inoculated (control).	Sown 21-8-07.	Ditto.	Ditto.
About 100 plants of I. sumatrana in 6 pots.	Inoculated with Neocosmospora from I. sumatrana.	Sown and inoculated 2-9-07.	No deaths up to March 28th, '08.	Ditto in 2 pots.
Ditto.	Not inoculated (control).	Sown 2-9-07.	Ditto.	Ditto.
60 plants of I. arrecta from Java seed in 4 pots.	Inoculated with Neocosmospora from I. arrecta.	Sown and inoculated 17-4-08.	No deaths up to March, '09.	
50 ditto.	Not inoculated (control).	Sown 17-4-08.	Ditto.	
58 plants of I. arrecta from Natal seed in 4 pots.	Inoculated with Neocosmospora from I. arrecta.	Sown and inoculated 17-4-08.	Ditto. Ditto.	
About 100 ditto.	Not inoculated (control).	Sown 17-4-08.	Ditto.	
11 plants of I. arrecta in one of the control pots of the third experiment above.	Inoculated with Neocosmospora from cotton.	Sown 17-4-08. Inoculated 9-7-08.	Ditto.	

No. of plants and variety.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
25 plants of I. arrecta in 2 of the control pots of the third experiment above.	Inoculated with Neocosmospora from gram.	Sown 17-4-08. Inoculated 3-11-08.	No deaths up to March, '09.	
About 20 plants of I. sumatrana in 1 pot and about 25 plants of I. arrecta from Java seed in 2 pots.	Sown in soil previously inoculated with pigeon-pea Neocosmospora.	Sown 15-11-08.	No deaths up to July, '08.	
About 20 plants of I. sumatrana in pot.	Ditto.	Sown 21-8-07.	No deaths up to November 25th, '07.	

This series appears to give conclusive evidence that Neocosmospora vasinfecta, though common on the roots of wilted indigo plants, is a pure saprophyte on them.

Gram Wilt.

The wilt of this crop (Cicer arietinum) occurs in Bengal, the United Provinces and the North-West Frontier Province. It is a typical wilt, having all the characters of the Fusarium wilts previously described. A severe outbreak was reported from Bannu on the North-West Frontier, and the specimens received in June, 1907, bore numerous perithecia of Neocosmospora on the roots. The wood of the coller and main roots was blackened and on section was found permeated by hyphæ. Microconidia (Cephalosporium) and macroconidia (Fusarium) were found as in the previous cases.

It is highly probable that the disease is a Fusarium wilt.

Series XIII.

(*a*) *Gram inoculated with Neocosmospora vasinfecta from gram.*

The cultures were part of the regular series mentioned above under Series X and bore numerous perithecia.

The plants were grown in pots, pots, soil and seed being sterilised in the usual way. The first sowing was made in July,

but all the plants, inoculated as well as control, died from heat, gram being a cold weather crop in India. They were resown at the beginning of the cold weather. At the same time a second series of inoculations was carried out, the sterilisation being very carefully attended to. The seed used in both cases was local seed, as wilt occurs sometimes in the local crop.

The inoculations were made in the usual way by sprinkling the seed and soil after sowing, but before covering, with the culture broken up in distilled water.

(*b*) *Gram inoculated with Neocosmospora vasinfecta from cotton.*

The culture was one of the regular series as before.

The plants were grown in pots and sterilisation carried out as before. As in (*a*) the first sowings were killed by heat and resowings made at the beginning of the cold weather.

The inoculations were as usual.

(*c*) *Gram inoculated with Neocosmospora vasinfecta from indigo.*

The cultures used were part of a regular series maintained from the second culture mentioned under Series XII, *i.e.*, that derived from perithecia on the roots of Indigofera arrecta.

The plants were grown and sterilisation effected as in (*a*) and (*b*) above.

The inoculations were as usual.

(*d*) *Gram inoculated with Neocosmospora vasinfecta from pigeon-pea.*

The culture was the same as that used in Series IX above.

The plants were grown, sterilisation effected and inoculations made as in (*a*), (*b*) and (*c*) above.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.
57 plants in 4 pots.	Inoculated with Neocosmospora from gram.	Sown and inoculated 1-11-08.	No deaths up to 5-3-09.
36 plants in 2 pots.	Not inoculated (control).	Sown 1-11-08.	Ditto.
44 plants in 2 pots.	Inoculated with Neocosmospora from gram.	Sown and inoculated 7-7-08. Resown 3-11-08.	Ditto.
30 plants in 2 pots.	Not inoculated (control).	Sown 7-7-08. Resown 3-11-08.	Ditto.
30 plants in 2 pots.	Inoculated with Neocosmospora from cotton.	Sown and inoculated 7-7 08. Resown 3-11-08.	Ditto.
25 plants in 2 pots.	Inoculated with Neocosmospora from indigo.	Ditto.	Ditto.
23 plants in 2 pots.	Inoculated with Neocosmospora from pigeon-pea.	Sown and inoculated 3-11-08.	Ditto.

These experiments show quite as conclusively as Series XI and XII, that Neocosmospora vasinfecta, though common on the roots of wilted plants, is purely saprophytic.

Summary of experiments with Neocosmospora vasinfecta Smith.

To sum up the experiments described under Series V to XIII above, the parasitism of Neocosmospora vasinfecta has been tested on four of the six crops hitherto found in India to be subject to wilt, and with this fungus common on the dead roots. These are pigeon-pea, cotton, indigo and gram. The diseases in pigeon-pea, cotton and gram are very similar, not only in their field characters and in their morbid anatomy, but also in the characters of the organism presumably the cause of the disease. This is a fungus with usually hyaline but occasionally brownish hyphæ, found predominantly in the xylem vessels but also penetrating the other tissues. In the vessels it often forms dense masses of matted hyphæ, which almost fill the lumen of the tube and must, of themselves, offer a considerable impediment to the flow of the ascending current. This stoppage is largely increased by a gummy exudation into the vessels, which commonly results from the presence of the fungus. On short lateral branches within the larger vessels, and also on hyphæ at the surface of the root, microconidia are formed. These are usually

formed singly when within the vessels, but in culture, or at the surface of the root in moist air, several are successively produced and remain bound together into a head by a watery or slightly viscid exudation from the tip of the sporophore. They thus belong to the form-genus Cephalosporium. On the surface of diseased roots and the base of the stem, beds of macroconidia of the type characteristic of the form-genus Fusarium are found. Fusarium spores, produced in this case singly, may also be derived from cultures of the microconidia, whether from within the vessels or from the bark.

On the dead roots, especially after incubation in a moist atmosphere, perithecia of Neocosmospora vasinfecta can frequently be found. From the ascospores of these, micro- and macroconidia, indistinguishable from those just mentioned, can be obtained by cultivation. In some cases wilted pigeon-pea stems also bear perithecia of a Nectria, which again has micro- and macroconidia similar to the above. The indigo disease is in some respects (which need not be entered on here) somewhat unlike the others. Here also, however, the morbid anatomy is similar, and the same micro- and macroconidia and perithecia of Neocosmospora are common.

The experiments described above resulted in not a single case of wilt being developed in cotton, indigo or gram when inoculated with cultures originating from the ascospores of Neocosmospora vasinfecta, whether from the same host or from any of the other three. In pigeon-pea apparent infections resulted in some cases, but these can all be accounted for by accidental inoculation from some other source and were not obtained when sterilisation of all the material used was rigidly carried out. The pigeon-pea wilt is very prevalent both on the Pusa Farm and in the surrounding neighbourhood, and sources of external infection are numerous and difficult to exclude. On the other hand, the cotton wilt has not been observed in this district and the danger of external contamination is negligible. Indigo wilt has occurred on the Farm, but there was little of it either on the Farm or near by, and the same applies to gram

wilt, so that in neither of these cases was the danger of accidental infection considerable.

Taking the whole of the experiments together, the conclusion is irresistible that Neocosmospora vasinfecta is a common soil saprophyte, which develops its perfect form on rotting roots of several (perhaps many) different plants and is wholly unconnected with the wilt diseases of the particular plants investigated so far in India. One might be tempted to go further and state that there is considerable doubt that the fungus is parasitic even in the United States. It seems at any rate clear that experiments intended to demonstrate the parasitism of polymorphic species of Nectria or Neocosmospora are useless unless carried out with pure cultures derived unmistakably from the perfect form of the fungus, and so far as I know, this has not previously been done in the case of Neocosmospora.

There are two questions which must now be answered. First, what evidence is there that the Indian Neocosmospora is identical with that of the United States, and second, if Neocosmospora is not the cause of wilt, what is the true cause ? In answer to the first I shall give a rather detailed account of the morphology and cultural characters of the Indian fungus which will leave no doubt as to its identity with that of the United States.

The second question can as yet only be answered in one of the four wilts referred to above, that of pigeon-pea. The ground has, however, been cleared for the study of the cotton and gram wilts, and I hope that it will be possible to ascertain definitely the cause of these diseases. The indigo disease is likely to prove more difficult, but a remarkable diminution in its intensity in the current season gives some ground for the hope that it may prove a passing outbreak of some half parasite, whose virulence may wane as the Java variety of plant gets acclimatised in its new home.

The identity of the Indian and American Neocosmospora.

Macroscopically and microscopically no difference can be detected between Neocosmospora vasinfecta from the United States

and the Indian species. The description and illustrations of the former in Smith's paper(2) are so full as to leave little to be desired for purposes of comparison.

The perithecia are of the same tone of bright red in both cases as a comparison of plate I of Smith's paper, and plate I below will show. They are formed, in India as in America, on the roots below ground, rarely above ground, and are quite superficial. Smith gives their size as from 210 to 400μ high, by 150 to 328μ broad, mostly 250 to 350 by 200 to 300μ. About the same limits have been found in the Indian forms, the most usual being from 250 to 350μ high by 175 to 280μ broad. The surface is almost smooth in both, being slightly irregular from the projection of individual cells only, not groups of cells as in many Nectrias. The neck is usually short, that figured in plate II, fig. 9 (cotton Neocosmospora), where it is about $\frac{1}{8}$th of the total height of the perithecium, being perhaps the most common type. Sometimes it is longer (plate II, figs. 7 and 8), occasionally quite long as in plate I, fig. 3. Smith gives 30 to 40μ as the usual length (or rather more than 1/10th of the total height), but says it may reach 80μ. I have found the greatest variability in this respect even in the same culture.

The asci are numerous, cylindrical, shortly stipitate, the spore-bearing part measuring from 70 to 100 by 12μ. They dissolve so as to liberate the spores within the perithecium when over-ripe. Paraphyses are present and consist of a chain of several rather large, irregularly oval, thin-walled cells, which are loosely connected. The figures in plate V, fig. 2, of Smith, and plate I, fig. 5, below, may be compared to show these characters.

The ascospores are eight in each ascus, arranged in one row, globose to shortly elliptical, and with a brown thick wall of peculiar construction. This consists of an outer layer which is irregular in thickness and often wrinkled, giving the mature spore an irregular contour, and an inner layer of moderate and quite regular thickness. These two layers are not tightly fused together, as macerated or ruptured spores show. Viewed in surface focus, the wall appears as if

pitted (the appearance is shown in an exaggerated form in plate I, fig. 4), but I have not been able to satisfy myself as to the exact structure of the markings seen, and they are not visible in optical section which tells against their being pits. Immature spores are colourless and with the outer wall little developed. The spores measure 10 to 15·5 by 10 to 15μ, usually about 12μ, if globose. The average of 100 spores from an agar culture of the cotton fungus gave 13·5 by 12μ. Fifty-four spores measured directly from pigeon-pea gave 10 to 14·5 by 10 to 12·5μ, the average being 11·9 by 11·5μ. Smith gives 10 to 12μ, if globose, and 8 to 12 by 11 to 14μ, if ellipsoidal, and notes that spores from a potato culture of the cow-pea fungus were generally larger than 12μ in diameter, and also larger than those measured directly from the host plant. In all the ascospore characters there is the closest possible agreement between the Indian and American forms, if we except the slight markings of the spore wall, which are not mentioned by Smith. They are only visible under high powers and are sometimes very difficult to make out, so that much importance does not attach to them.

The conidial stages are, as pointed out above, practically valueless for purposes of comparison. Their variability is such that it is hardly possible to separate by microscopic examination and measurements alone many of the different species of Fusarium from one another, and still less those of Cephalosporium. Smith gives the spores of the former stage of Neocosmospora vasinfecta as lunulate, 3 to 5-septate, and 30 to 50 by 4 to 6μ in diameter, and of the latter as oval to narrowly elliptical, straight or slightly curved and 4 to 25 by 2 to 6μ. I have found that the majority of the Fusarium spores are from 35 to 40μ long, but the lower limit is very elastic, as all gradations down to the Cephalosporium type can be found. The breadth of the spores and the number of septa agree with the data given by Smith, but the intermediate types between Fusarium and Cephalosporium often have one or two septa. These types develop chiefly in culture, the spores formed in stromata on the bark of the host being more regular. The Cephalosporium spores average about 6 to 10 by 2 to 4μ, and

their mode of formation is shown in fig. 5, plate II, which may be compared with fig. 3, plate III of Smith's paper.

Chlamydospores are not uncommon in the vessels of old diseased roots, and are also formed on the surface and in old cultures. They are figured on plate II, fig. 4, which may be compared with fig. 12, plate III of Smith.

The cultural characters of Neocosmospora vasinfecta are very fully described by Smith. The greater part of his observations relating to pigment production were, however, carried out with cultures of the watermelon fungus originating from conidial forms on the host, not from ascospores ; there is, therefore, no certainty that they belonged to Neocosmospora. They never developed perithecia though continued for five years on a variety of media. On the other hand, his observations regarding the influence of different media on perithecia formation were made with ascosporal strains of the Cow-pea Neocosmospora. The following table gives a comparison between some of the results noted by Smith and those observed in ascosporal cultures of the Indian fungus from pigeon-pea, indigo and cotton.

Cultural characters of American and Indian Neocosmospora from different hosts.

Cow-pea (Smith).	Pigeon-pea.	Indigo.	Cotton.
Steamed potato. 6th day, colour of substratum dark lead grey. Perithecia roseate by 6th to 9th day, then bright coral red.	*Steamed potato.* In about a week surface growth pink, no violet or purple. Below a pale green stromatic line. Brown stroma in contact with glass. Perithecia light red. 14 days, perithecia darker, some brown.	*Steamed potato.* 7 days, growth white to dark greenish grey. Perithecia numerous, scattered, pale red to deep red. 16 days, mycelium chiefly dark grey. Perithecia mostly rose.	*Steamed potato.* 7 days, flocculent, growth white, stromata scanty pale green to pale purple. 16 days, perithecia many, bright red.
Pearl tapioca. 8th day, not 1/1000th as much growth as on potato. Perithecia few, appear slowly, at first pale red, then black. Remain sterile.	*Tapioca.* 14 days, no growth.	*Tapioca.* 7 days, no growth. 16 days, growth hardly visible. No perithecia.	*Tapioca.* 7 days, no growth, 16 days growth scanty. No perithecia.

Cow-pea (Smith).	Pigeon-pea.	Indigo.	Cotton.
Banana. Groth slow and perithecia appear after some weeks, then many, pale yellowish red or ochraceous buff after 20 days. After 2 months one culture with red perithecia, and fertile, others with dark brown or black and sterile.	*Plantain.* 7 days, growth good, white tinged with pink. No perithecia. 34 days, perithecia visible, dark red.	*Plantain.* 7 days, growth less than on potato, white to grey. Perithecia few, pale. 16 days, perithecia yellow, a few pink.	*Plantain.* 16 days, growth not good, no perithecia.
Spanish Onion. Perithecia appear slowly, then many, pale red to bright coral.	*Onion.* 7 days, growth pinkish, flocculent, numerous perithecia, light red. 14 days, perithecia darker.	*Onion.* 7 days, growth white to greenish, no perithecia. 16 days, numerous bright red perithecia.	*Onion.* 7 days, growth white, no perithecia. 16 days, perithecia rather scanty, pale.
Acid rice. (5 drops very dilute HCl added to each tube). 16 days, substratum dark purplish red. Perithecia numerous, full grown, colourless to yellowish red. No bright red.	*Acid rice.* (5 drops ½% HCl added to about 10 c.cs. boiled rice). 7 days, growth feeble, upper part pinkish. 14 days, colour deeper, no perithecia. 34 days, numerous perithecia.	*Acid rice.* (As in column 2). 7 days, growth dark purple or claret, perithecia many, yellow to black. 16 days, perithecia mostly dark purple, none red.	*Acid rice.* (As in column 2). 7 days, growth copious, pale pink to bluish-purple. 16 days, mostly pink, no perithecia. With more strongly acid rice (10 drops acid), a few pale perithecia appeared after 56 days.
Alkaline rice. (10 c.cs. rather dry boiled rice, with 2 c.cs. cold saturated solution bicarbonate of soda). After 40 days growth sparing, no perithecia.	*Alkaline rice.* (5 drops saturated solution of sodium bicarbonate added to about 10 c.cs. of boiled rice), 7 days, growth moderate, stromata dark brown, no perithecia. 14 days, a few light red perithecia.	*Alkaline rice.* (As in column 2). 7 days, growth greenish to brownish, no perithecia. 16 days, mycelium almost black above, yellow to brown below, no perithecia.	*Alkaline rice.* (As in column 2). 7 days, growth white, no perithecia. 16 days, lower part dirty green, no perithecia. 56 days, a few light red perithecia.

The characters given above agree particularly closely in the case of the cow-pea fungus of Smith and the indigo fungus. In the other cases some divergencies occur, but the general behaviour corresponds fairly well. The differences, such as they are, are as general between the three Indian strains as between these and the American, and are such as are inseparable with working with media of the indefinite composition of those mentioned.

No one could think of separating as distinct species, fungi agreeing so generally in their microscopic characters and cultural behaviour as the Neocosmosporas studied in the United States and in India.

CHAPTER III.

Fusarium udum n. sp., the cause of pigeon-pea wilt.

The repeated examination of the roots of wilted pigeon-pea plants in all stages of the disease, carried on during the course of the investigations described above, left practically no doubt that the disease was a typical Fusarium wilt. Attempts to prove its connection with Fusarium stages of the two Hypocreaceous fungi found on the bark, the Nectria found at Dehra Dun and Neocosmospora vasinfecta, having failed, it remained to look elsewhere.

The number of soil organisms that may be obtained from dead vegetable matter in the soil (such as the surface of the roots of a dead or dying plant) is greater than would be anticipated by any one not accustomed to the specific examination of soils for fungi. In any locality several are sure to be so common as to be found on almost any root examined, and amongst these are Fusaria probably belonging to several different species. It appeared an endless matter to hunt through these for possible parasites. If, however, the superficial forms could be excluded or destroyed, it seemed likely that the true cause could be induced to give a comparatively pure surface growth by appropriate treatment. The following procedure was, therefore, adopted to isolate any truly internal organisms that might occur in diseased roots.

A short length of tap root from a just wilted seedling was cut off with a flamed scissors and the presence of the internal mycelium determined by sectioning the end of one or other of the rejected pieces. The root was washed thoroughly, first in tap water and then in several changes of distilled water, being well rubbed between the fingers to remove irregularities where particles of soil might lodge. It was then seized in sterile forceps, dipped in absolute alcohol for about 5 seconds, the alcohol flamed off and the root

rapidly dropped into a tube of sterile prepared medium. In this manner it was hoped to destroy all but organisms deep in the tissues. All the roots thus treated gave rise to the growth of a bacillus which gave characteristic colonies on nutrient agar. In some the fungus known to be present within the root was killed by the treatment, and no mycelial growth occurred. In others a filamentous fungus appeared on the surface or at the cut ends, and was easily got into pure culture free from the bacillus. Only these two organisms, a bacillus and a fungus, were thus obtained and work was continued with them.

Series XIV.

The culture was obtained from a wilted pigeon-pea root grown in soil which in part originated from pot V of Series V above (p. 10). On August 21st, 1907, all the plants in this pot had wilted and were pulled out. The soil was then mixed with some fresh potting earth in equal parts, and filled into two pots. In these pigeon-pea and indigo respectively were sown. Wilt appeared in the former on October 10th, and all the plants were dead by November 21st. They were pulled out and perithecia of Neocosmospora developed on some of them after 10 days in a moist chamber. Pigeon-pea was resown in the pot immediately. On November 25th, the indigo plants in the other pot (still perfectly healthy) were pulled out and pigeon-pea sown in the pot, after adding as usual an equal bulk of fresh potting soil. The pigeon-pea after pigeon-pea developed wilt during January, 1908, and that after indigo at the end of the same month. Eight plants in each pot were dead by March 28th. The rest were then pulled out and the soil from the two pots mixed, part being used to grow yet another pot of pigeon-pea. This showed wilt in a month, being the fourth generation of seedlings that had succumbed to the infective material of pot V.

On May 19th, 1908, a very recently infected root was selected from a wilted plant of the last batch, washed in water and alcohol and flamed as described above, and dropped into a tube of nutrient agar. On the 2nd day the contents of the tube, now clearly showing a growth of hyphæ and bacteria around the root, were plated

and the fungus transferred in pure culture from a single-spore colony to an agar tube three days later. The resulting growth differed considerably in appearance from any stage of Neocosmospora, though on examination it was found to consist of Cephalosporium microconidia. Subcultures from this tube on steamed carrot and potato were used for the inoculations below.

The plants were grown in pots sterilised by immersion in ·001 per cent. corrosive sublimate for about 5 minutes before filling with soil. The soil was sterilised by intermittent steaming on three days, the internal mass being maintained at about 87° C. for two or three hours each day. The seeds were sterilised as usual by steeping in ·1 per cent. formaldehyde for 2 hours. About 25 plants grew in each pot.

The inoculations were made by sprinkling the seeds and soil with the cultures broken up in distilled water, after sowing but before covering.

No. of pot.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
1	Inoculated with Cephalosporium from pigeon-pea.	Sown and inoculated 13-6-08.	Wilt appeared.	1st death on 16-7-08, typical wilt.
2	Ditto	Ditto	Ditto	1st death on 1-8-08, typical wilt.
3	Ditto	Ditto	Ditto	1st death on 12-8-08, typical wilt.
4	Ditto	Ditto	Ditto	1st death on 19-7-08, typical wilt.
5	Not inoculated (control).	Sown 13-6-08.	No death up to September 4th, '08.	...
6	Ditto	Ditto	Ditto	...

All the plants, except one, in the four inoculated pots were dead by October 26th. On this date those left were pulled out and examined. The number of dead plants in the four pots was 18, 18, 16 and 19 respectively, several in each pot having been pulled out for examination on previous occasions. Most had no surface growth of fungi on the roots, though about one-third showed whitish patches with single-celled spores on a fine mycelium resembling Cephalosporium. Several were placed in the moist chamber and gave rise to Cephalosporium and in some cases also Fusarium after some days. None gave perithecia, nor were any observed on any plant, whereas those that had wilted in soil taken from pot V of Series V developed perithecia of Neocosmospora vasinfecta when similarly incubated. This series, therefore, combined with Series VI above, showed that the soil of pot V contained a harmless fungus, Neocosmospora vasinfecta, with which it had been inoculated, and a virulent parasite, the fungus now under consideration, which must have entered accidentally either from the pot walls, the seed or the air.

A macroscopic and microscopic examination of the diseased roots was carried out. The characters of the wilt and the gross appearance of the tissues were found to be exactly similar to those of cases of wilt occurring naturally on the Farm. In section the conditions were just as described in my previous paper on this disease, the hyphæ being found chiefly in the vessels, where also microconidia and chlamydospores were observed. As this experiment coincided in time with the gram inoculations mentioned under Series XIII, in which plants inoculated with Neocosmospora (as well as the controls of the same series) died, the death being from heat and not from Neocosmospora as was proved by the further sowings in the same pots later in the year, when the temperature had fallen, a comparison was made between the roots in the two cases. In both the conditions were sufficiently alike to lead to considerable difficulty in distinguishing between them. The gram roots were permeated with the hyphæ of Neocosmospora which attained their chief development in the vessels. On the surface, while still in the soil,

or after some days in the moist chamber, nearly all the gram plants developed perithecia of Neocosmospora. From this it is apparent that Neocosmospora, though a saprophyte, penetrates freely into roots killed from other causes and fills the vessels with a mycelium closely resembling that of the true wilt fungus.

Series XV.

The culture used was obtained from the third batch of plants grown in the soil from pot V of Series V. A very recently attacked root was selected, washed carefully in several changes of distilled water and rubbed well between the sterilised fingers to remove adhering soil and dead bark. It was then placed in distilled water on a sterile slide. In 24 hours a fine growth was visible on the surface and a trace of this aerial mycelium was transferred to an agar tube. On the 6th day there was a distinct growth along the streak and a subculture was made from one point on this to another agar tube. This proved microscopically pure and was used for the inoculations after 13 days.

The plants were grown in pots, twenty-five seeds being sown in each.

The seeds, soil and pots were sterilised and the inoculations made as in Series XIV.

No. of pot.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
1	Inoculated with Cephalosporium from pigeon-pea.	Sown and inoculated 17-4-08.	Wilt appeared.	1st death on 6-6-08, all dead by 12-9-08.
2	Ditto.	Ditto.	Ditto.	1st death on 12-5-08, all dead by 8-9-08.
3	Not inoculated (control).	Sown on 17-4-08.	No death up to September, '08.	..
4	Ditto.	Ditto.	Ditto	...

Here, again, the wilt was typical and virulent. All the plants (nearly 50) in the inoculated pots were dead by the early part of September, the control plants remaining perfectly healthy.

Series XVI.

The cultures were obtained as in Series XV, and were grown on carrot, onion, plantain and rice.

The plants were grown in a small plot in my garden, 8 by 4 feet in size, the seeds being steeped in formaldehyde as before.

The inoculations were made as in the last two series.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
Contents of a plot 8×4 feet.	Inoculated with Cephalosporium from pigeon-pea.	Sown and inoculated 1-7-08.	Wilt appeared.	1st death on 10-8-08. A large number were dead by 26-10-08.
Ditto	Not inoculated (control).	Sown on 1-7-08.	No deaths up to March, '09.	...

The two plots were side by side, separated by a small trench about a foot in depth. It is somewhat remarkable that there was no spread from the inoculated to the control part by aerial conidia, as other experiments seem to show that such a spread occurs not uncommonly in nature.

Series XVII.

The cultures used were the same as in the last series.

The plants were two clumps over a year old, growing in open soil in my garden, and were the contents of pot No. 3 (inoculated previously with Neocosmospora) and pot No. 7 (not previously inoculated) of Series V above (page 10). These had been cut back when about 9 months old and had given a second vigorous growth, being still perfectly healthy.

The inoculations were made by scraping away the soil down to the finer roots and burying part of the culture in contact with the latter.

No. of plants.	Treatment.	Date of inoculation.	Result.	Remarks.
About 5 plants out of a clump of about a dozen which had been a year previously inoculated with Neocosmospora.	Inoculated with Cephalosporium from pigeon-pea.	Inoculated 1-7-08.	Wilt appeared.	The first death was on 29-7-08. All in the clump were dead by the end of the year.
The same in a clump not previously inoculated.	Ditto.	Ditto.	Ditto.	The first death was on 24-7-08. All were dead by November, '08.

The disease spread from the inoculated clumps to those belonging to pots 2, 4, 6, 8, 9, 10, 11 and 12 of Series V, all of which were near together and not separated by any obstacle from the inoculated clumps, and were perfectly healthy at the time of inoculation. Hence, though the plants from pot 3 had flourished for more than a year after being inoculated with Neocosmospora, they succumbed without exception to the attack of the Cephalosporium in from one to five months, though they were very large plants, mostly over 8 feet high.

The proof is therefore complete that the Cephalosporium isolated from the roots of wilted pigeon-pea plants in the manner described under Series XIV above, is a virulent parasite and that it, and not Neocosmospora, is the cause of the wilt disease commonly found in this crop.

Series XVIII.

An experiment was carried out to test whether the presence of Neocosmospora in any way altered the course of the disease induced by the Cephalosporium isolated from the interior of wilted pigeon-pea roots.

The culture of the Cephalosporium used was a subculture on steamed potato from that used in Series XIV. That of Neocosmospora was an agar subculture originating from the culture on pigeon-pea used to inoculate pot 2 of Series V above.

The plants were grown in pots; pots, soil and seeds being sterilised as in Series XIV, and the inoculations were made as in that Series.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
About 25 plants in 2 pots.	Inoculated with Cephalosporium isolated from the interior of pigeon-pea roots combined with Neocosmospora from the surface of the roots.	Sown and inoculated 18-6-08.	Wilt appeared.	The first death was on 24-7-08.
Ditto.	Not inoculated (control).	Sown 13-6-08.	No deaths up to 4-9-08.	...

The presence of Neocosmospora appeared to make no difference in the rapidity of onset or characters of the disease.

As mentioned on page 53 above, a bacillus was constantly obtained from the interior of the roots of pigeon-pea plants affected by wilt, after flaming. The action of this bacillus on healthy plants, both alone and in conjunction with Neocosmospora and the parasitic Cephalosporium, was tested.

Series XIX.

The culture of the bacillus used was obtained as described under Series XIV above and isolated by plating. That of the Neocosmospora was the same as in Series IX. That of the parasitic Cephalosporium the same as in Series XIV.

The plants were grown in pots; pots, seeds and soil being sterilised as in Series IX and XIV, and the inoculations made as in those Series.

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
About 25 plants in 2 pots.	Inoculated with bacillus isolated from the interior of wilted pigeon-pea roots.	Sown and inoculated 13-6-08.	No deaths up to 2-9-08.	.....

No. of plants.	Treatment.	Date of sowing and inoculation.	Result.	Remarks.
About 25 plants in 2 pots.	Not inoculated (control).	Sown 13-6-08.	No deaths up to 4-9-08.	.....
22 plants in 2 pots.	Inoculated with a mixture of the above bacillus and Neocosmospora from pigeon-pea.	Sown and inoculated 1-11-08.	No deaths up to 16-3-09.	.....
About 25 plants in 2 pots.	Inoculated with a mixture of the above bacillus and the parasitic Cephalosporium.	Sown and inoculated 13-6-08.	Wilt appeared.	1st death on 10-7-08. All were dead by October, '08.

The bacillus itself or in conjunction with Neocosmospora is unable to cause disease. When combined with the parasitic Cephalosporium it causes no apparent change in the rapidity of appearance and nature of the disease caused by the latter. It has not been seen in the tissues and so presumably is present in small numbers. A quite similar bacillus was obtained from the roots of healthy jute plants in the same manner. What part these organisms play when present in such roots is unexplained.

In all these inoculation experiments, carried out with no less than four distinct organisms found more or less regularly associated with the disease, one and one only of the four has been found definitely parasitic. The other three are harmless. Were it not that two of these three possess stages in their life-history closely resembling the true parasite, and frequently occur intermingled with it, there would have been no need for the laborious testing in pure separate cultures of the various organisms. It appears clear that much of the work that has been done with Fusarium wilts has met with the same difficulties, and in so far as these difficulties have not been allowed for, so to the same extent the work is unreliable. It may be said definitely that no case of parasitism attributed to Fusarium or Cephalosporium stages of Hypocreaceous fungi can be accepted unless successful inoculations have been carried out with ascosporal strains. Similarly none of these conidial

types of soil fungi can be considered a proved parasite unless successful inoculations have been obtained in each case with pure cultures of the fungus concerned. The mere presence of a fungus in the tissues of the diseased plant with conidial stages agreeing with those of a recognised parasitic form of Cephalosporium or Fusarium counts for nothing without successful cultivation and inoculation.

Description of the parasite of pigeon-pea wilt.

The characters of the internal growth in diseased roots have been described in the previous paper. When such roots are well washed, flamed and placed in water on a slide, an external growth appears on the surface in a day or two. This has at first consisted only of the Cephalosporium type in the cases observed, but subsequently Fusarium spores appear. The Cephalosporium is characterised by a rather scanty creeping mycelium, with very many short conidiophores, often whorled, and bearing great numbers of microconidia. There is ordinarily little tendency for the growth to extend into the air ; most of the conidiophores remain immersed in the liquid and therefore do not show the characteristic heads of spores bound in a drop of water. These occur, however, in cultures on solid media and may be studied in agar drop cultures on slides or in glass cells (plate IV, fig. 3).

The following description is taken from pure strong cultures of the fungus, the parent of which or subcultures from which have been proved to be parasitic by inoculation. The cultural characters on different media will be given in detail below.

The hyphæ are fine, copiously branched, septate and colourless under the microscope. Anastomoses occur rarely in vigorous cultures, but are common when a portion of the growth in an old culture is sown in water, and also between the germ tubes of both sorts of conidia (plate IV, fig. 10). Cephalosporium spores begin to form when the mycelial growth is still scanty, and are found in great numbers by the third day in most good cultures. Singly they are

colourless but in the mass range from flesh colour to deep salmon pink on most media. They give a peculiar moist, almost oily, appearance to the surface of the growth from their great numbers. They are formed either on single conidiophores arising directly from the creeping mycelium (plate IV, fig. 2), or on special whorled branches in dense clusters (fig. 1). If there is any morphological character which distinguishes this species from its allies, it is this formation of crowded whorled conidiophores. I have not observed them in other species seen. They are not, however, always formed and the other type, which is always present, is not distinguishable from that of several allied species.

Even at an early stage of the cultures septate spores are formed. The typical forms found at this period are straight or slightly curved, continuous and measure 5 to 15 by 2 to 4μ. The other forms which occur mingled with these are falcate or strongly lunulate, single-celled spores, larger than the last (up to 21μ in length), and one, two or sometimes three-celled spores, which may be straight or curved and with pointed or rounded ends. Examples of all the types seen in a single culture on the third day are shown on plate IV, fig. 4.

In a few cultures on potato and plantain, small round sclerotia, bearing microconidia, appeared. These seem to correspond to the Tubercularia stage of Nectria cinnabarina.

Many of the cultures, especially some of those on liquid media, formed no other kind of spore but the above. In others Fusarium spores develop. They appear later than the other forms and are figured in plate IV, fig. 7. Quite similar spores may be got from any culture by inoculating a drop of agar on a slide, and it appears probable that they occur regularly in all good solid-medium cultures that are well aerated, but less frequently in liquid media or in badly aerated cultures.

The Fusarium type of spore may be formed either singly on short lateral conidiophores, or in clusters. A true tuberculariaceous stroma bearing macroconidia has not developed in any of the

cultures, but it almost certainly does so in nature on the bark of the host plant. I have not been any more successful in the prolonged cultures of Neocosmospora on a large variety of media in obtaining conidial stromata. Such stromatic beds are common on the bark of wilted plants and some, at least, would appear to belong to the parasite.

The Fusarium conidia are formed in a manner quite like that of the microconidia, except that the spores successively abjuncted from the apex of the conidiophore, drop off at once, and are not bound in a drop of water into a head. They are falcate or more rarely nearly straight, with pointed ends, 3 to 5-septate, slightly swollen between the septa and measure 30 to 50 by 3 to 5μ.

In several cultures, particularly on potato, a second type of Fusarium spore developed. This was nearly as long as the first but much narrower and produced on whorled conidiophores resembling the clustered type often found in the microconidial stage.

Both micro- and macroconidia germinate readily in water, putting out one or more germ tubes, which may grow out into a mycelium or may be of limited growth and give rise to secondary spores, usually of the microconidial type, but sometimes of the other. Several of those figured in fig. 7, plate IV, arose from the germ hyphæ of the microconidia figured in fig. 6 of the same plate.

Chlamydospores are found on the mycelium within the vessels and also develop in culture. They are usually round or oval swellings of a hypha, either at the end or in its length, and are sometimes in short chains of two or three. The wall is thick but not, so far as seen, coloured, in this differing from the similar bodies of several of the common soil Fusaria. In old cultures portions of the mycelium may become segmented into thick-walled, irregular cells, which appear to be of the same nature as chlamydospores.

The above description is not sufficient to separate this species from conidial stages of more than one species of common soil fungi. In comparing it with conidial stages of Neocosmospora vasinfecta the only character which appears to be of diagnostic value is the

clustered conidiophore of the microconidial stage, which, however, is not a constant character in culture. The species named Fusarium Dianthi Pril. and Del.(17), the cause of the carnation disease in the south of France, is evidently closely similar to the conidial stages of Neocosmospora, since Delacroix in a subsequent paper(5) stated that they appeared to be identical. This same fungus was referred by Mangin(18) to Fusarium roseum, one of the commonest members of the genus, and hence this must also be considered as a close ally of the pigeon-pea parasite. Similarly, Fusarium oxysporum Schlect., with its numerous possible synonyms, is hardly to be distinguished morphologically from this species except in the more regular, coloured chlamydospores. The latter are, however, very variable bodies. Fusarium Lini Bolley (19), another of the proved wilt-producers, is also probably a close ally. It is true that Bolley does not specifically mention a microconidial stage, but from his figures on pages 37 and 45 it would appear probable that one exists.

In the absence of the perfect fruit it appears therefore necessary to rely on cultural characters for some of the chief diagnostic features of the pigeon-pea parasite.

Cultural characters of the pigeon-pea parasite.

The following media were inoculated from an agar tube culture originating from a single spore and being part of the material used for the successful inoculations in Series XIV.

Nos. 1 to 10 consisted of a standard solution made up of ammonium nitrate 10 grms., potassium phosphate 5 grms., magnesium sulphate 2·5 grms., and distilled water 1,000 c.cs., to which were added the substances specified below. The liquid cultures were in Pasteur flasks containing 30 c.cs. of the medium, the solid cultures in tubes.

A. Liquid media.

1. Glucose 3% added to the standard solution.
2. Saccharose 3% ,, ,, ,,

3. Maltose 3% added to the standard solution.
4. Glycerine 5% ,, ,, ,,
5. Gum acacia 5% ,, ,, ,,
6. Peptone 1% ,, ,, ,,
7. Asparagin 1% ,, ,, ,,
8. Citric acid 1·5% ,, ,, ,,
9. Sodium carbonate 1% ,, ,, ,,
10. Absolute alcohol ·5% ,, ,, ,,

B. Solid media

11. Potato boiled in distilled water.

12. Acid potato, prepared by soaking for 20 minutes in 1% sulphuric acid, then rinsing quickly in water and boiling in distilled water to sterilise.

13. Alkaline potato, as 12, but 1% sodium carbonate used in place of sulphuric acid.

14. Carrot boiled in distilled water.

15. Acid carrot, as 12.

16. Alkaline carrot, as 13.

17. Rice boiled in a paste with distilled water.

18. Acid rice, about 10 c.cs. rice moistened with distilled water and with 10 drops of ·5% hydrochloric acid added.

19. Acid rice, as 18, but only 5 drops acid added.

20. Alkaline rice, as 18, but 5 drops of saturated solution of sodium carbonate added in place of the acid.

21. Alkaline rice, as 20, but only 2 drops alkali added.

22. Plantain boiled in distilled water.

23. Onion boiled in distilled water.

24. Sugarcane pith boiled in distilled water.

25. Tapioca boiled in distilled water.

26. Nutrient agar; agar 15 grms., beef extract (Liebig's) 4 grms., peptone 10 grms., sodium chloride 5 grms., distilled water 1,000 c.cs., neutralised by sodium carbonate.

27. Glucose agar as last but with 20 grms., glucose added.

The characters of the growth on these media are given below.

1. *Standard solution + glucose.*—After a week the growth in this flask was ahead of that in any other of the series. The surface growth was dense and of a moist pink appearance from spore production. The deep growth entirely filled the solution. After 15 days this was still the best flask, the submerged growth being very dense. Neither in this nor in any other of the liquid media was there any appreciable aerial growth. After two months the surface colour had faded to a dirty flesh colour with some creamy patches. All three spore forms were produced.

2. *Standard solution + Saccharose.*—The growth was slower than with glucose, the surface growth after 15 days being only about one-half that of the latter. After two months the colour was a deep pink, much brighter than in 1. All three spore forms were produced.

3. *Standard solution + maltose.*—The growth was patchy at the surface though dense below after a week. The surface patches were salmon pink from spore formation. The latter had increased by the 15th day but still did not cover the whole surface as in 1 and 2. In two months the colour was intermediate between 1 and 2, but the growth still less than in either. All three spore forms were produced.

4. *Standard solution + glycerine.*—Growth fairly dense after a week but no colour. In 15 days the superficial growth was greyish white. In two months a little colour had appeared, and this gradually developed into a uniform pale creamy pink which persisted for many months. All three spore forms were produced.

5. *Standard solution + gum acacia.* — Growth of the same character as in glycerine but denser and the pink tinge appeared in 15 days, and was about No. 16 of Saccardo's "Chromotaxia" after wo months. Macroconidia not developed.

6. *Standard solution + peptone.*—Surface growth slight, white. Submerged growth less than in any of the above, flocculent, not in sheets. No colour developed after many months. Few spores were formed and all of the microconidial type.

7. *Standard solution + asparagin.*—Surface growth better and submerged growth worse than with peptone, after a week. Then improved and after 15 days the surface was nearly covered with a moist white growth with well defined margins and concentric, slightly raised, lines of growth. In two months all growth had apparently ceased. Spores not numerous, only microconidia.

8. *Standard solution + citric acid.*—The only growth visible after a week was a number of minute flocculent colonies. These increased subsequently but little and never developed any colour. A moderate number of all three kinds of spores developed.

9. *Standard solution + sodium carbonate.*—No visible growth at the end of two months.

10. *Standard solution + alcohol.*—No surface growth resulted at any time. The submerged growth was thin and filmy but fairly abundant. Microconidia abundant, macroconidia and chlamydospores few.

11. *Normal potato.*—After three days the surface was practically covered with a dense rough growth, grey or white at the base, but with dirty pink patches over a considerable portion. After two months the whole slab was covered with an even, moist, non-filamentous growth of a dirty cream colour. All the potato cultures bore eventually all three types of spore.

12. *Acid potato.*—After three days the whole slab was covered with a dense moist growth, mostly salmon pink from copious spore formation (see plate III, fig. 2). The growth in this tube remained one of the best of the series. After some months the upper part of the slab was nearly black from a development of thick-walled brittle hyphæ, with short segments and brown walls. A few small pink sclerotoid bodies, similar to those described under No. 22 below, were found after about four months.

13. *Alkaline potato.*—After three days the growth was in isolated white woolly tufts with no trace of colour and without the moist appearance of 12 (see plate III, fig. 1). A pink tinge

appeared about the 7th day and was well developed, though still paler than on acid potato, by the 12th day.

14. *Normal carrot.*—After 15 days the growth was good, colour pale salmon, surface moist, submerged growth in the water at the bottom of the tube colourless and flocculent. Spores very numerous, mostly slightly falcate, unicellular but also some with one or more septa. No macroconidia after a month.

15. *Acid carrot.*—Growth as in 14, but colour tint deeper in 15 days. Submerged growth in the water at the bottom of the tube in a dense pale-yellow sheet. Spores as in 14.

16. *Alkaline carrot.*—Growth as in 15.

17. *Normal rice.*—After 15 days the growth was considerable, surface colour salmon pink and dry, with numerous coremial-like strands extending to the glass, and copious spore-formation. Deep colour in contact with the substratum orange-red. This was the deepest colour produced in any culture. In the lower part of the tube, where aeration was deficient, the colour varied from salmon pink to pale orange.

18. *Acid rice.*—10 drops acid, after 15 days colour as 17 but more brick-red (see plate III, fig. 3).

19. *Acid rice.*—5 drops acid, not distinguishable from 18.

20 & 21. *Alkaline rice.*—5 and 2 drops alkali respectively, as 18 but growth less and colour paler.

22. *Plantain.*—After 15 days growth copious, woolly not moist, dirty flesh-colour, coremial strands as in No. 17. Where the growth was confined between the medium and the glass a thick yellow or white stroma formed. A surface sheet, almost colourless, covered the liquid in the bottom of the tube and on this several sclerotial bodies were formed. These consisted of pseudo-parenchyma, lined on the surface with crowded, erect, short conidiophores from which microconidia were budded off. The whole arrangement corresponded to the Tubercularia stage of some Nectrias. On the rest of the culture the spores were broader than usual.

23. *Onion.*—In one tube the growth resembled that on plantain, but the surface growth on the liquid below was brick-red in colour, and no sclerotia were formed. In another tube the growth was moist and pink as on normal potato and there were no coremial strands.

24. *Sugarcane pith.*—Growth very feeble and scarcely visible after a week. It ceased entirely soon after. A few microconidia only were formed.

25. *Tapioca.*—After 15 days growth slight and confined to the point of inoculation. Colour pale salmon pink. A few microconidia.

26. *Nutrient agar.*—In streak cultures the growth appears slowly, being visible on the third day, and forming a raised distinct band by the 6th day. The band is strongly convex, limited closely to the neighbourhood of the streak, and the rest of the surface of the agar long remains free from the fungus. There is usually little aerial growth, the streak being not unlike a bacterial growth. In the centre it is from salmon pink to brick-red, and around this is a zone of scanty superficial growth distinctly filamentous under a lens. The raised part of the streak is uniformly moist and shiny as soon as spore-formation becomes considerable. After about 2 weeks portions of the streak become greyish black and this may gradually extend to the whole growth. The spores are very variable even in young cultures, those figured on plate IV, fig. 4, being sketched from a single culture 3 days old. In old tubes macroconidia occur, but they are never numerous on this medium. Chlamydospores form on agar, as on most of the other media, after some months.

27. *Glucose agar.*—The addition of glucose does not appear to affect the character of the growth to any extent. It is rather more vigorous, and the black colour may develop more quickly.

In seeking for diagnostic characters in the above series of cultures, most weight naturally attaches to such characters as appear

more or less regularly in the media on which the fungus grows satisfactorily. Of these the most striking is the moist, bacterial-like surface growth which developed sooner or later in all the cultures in which growth was considerable, except rice and plantain. It is most strongly developed in ordinary agar streak cultures, which would be taken on a cursory examination for bacterial growths. It is quite unlike the growth of the conidial stages of either Neocosmospora vasinfecta or of the Nectria mentioned in the early part of this paper. The second character of importance is the colour. In almost all the successful cultures this developed first with spore-formation and was largely confined to the spores. It ranged from pale flesh colour to salmon pink in these cases, and examination of spores in the mass never indicated a deeper tone than the latter. On rice, however, the substratum and the mycelial layers in contact with it developed a much deeper orange-red (plate III, fig. 3). This was altogether independent of spore-formation, and examination showed that the colour was contained in the hyphal contents not in the walls. In no cases were any green, blue, purple or carmine tones developed and this fact serves at once to distinguish the species from Neocosmospora (*cf.* plate III, figs. 4 and 5).

Of less important characters, because less constant, the chief is the formation of Tubercularious stromata observed in cultures on acid potato and on plantain. The formation of coremial strands on rice, plantain and onion is a character of the same nature, but is not constant even on these media, as the onion cultures showed.

Of other cultural characters it may be said that the fungus grows equally well when carbohydrate is supplied as sugar (in the liquid cultures) or as starch (in the solid cultures). In the former case glucose is the most suitable, saccharose next and maltose least of the three tried. Similarly of starchy media potato, carrot, rice and plantain were the best in the order named, while tapioca was hardly capable of utilization at all. As regards reaction, acid potato, carrot and rice gave better results than normal, and in potato and rice the normal was better than the alkaline culture. In

carrot the two were about equal. Though in the standard solution with 1% sodium carbonate no growth took place, the tolerance for alkali is shown in the alkaline cultures on solid media and was also evident in some field experiments mentioned below, in which the disease was not diminished by heavy liming. Most Fusaria so far investigated agree in this respect.

For the fungus possessing the characters above described I propose the name Fusarium udum, without prejudice to the question, which I hold to be unanswerable, whether it has been previously included amongst the named members of the genus. The following diagnosis should serve to identify it with sufficient accuracy.

Fusarium udum n. sp.

Mycelium parasitic within the roots of the host plant or saprophytic and then creeping ; hyphæ hyaline, slender, much branched, usually with little aerial growth ; microconidia of the Cephalosporium type, produced successively on the ends of short simple or clustered conidiophores and remaining bound in a drop of liquid after abjunction, unicellular or with 1 or more septa, elliptical or falcate, hyaline singly, salmon pink in mass, occasionally developing from the surface of minute spherical stromata and then of the Tubercularia type, 5 to 15 by 2 to 4 μ in diameter ; microconidial stage in culture usually moist and bacteria-like, white to salmon pink, occasionally (on rice) orange-red, never green, blue or purple ; macroconidia of the Fusarium type, formed as the microconidia but on shorter conidiophores and becoming free as soon as abjuncted, falcate, 3 to 5-septate, hyaline, 15 to 50 by 3 to 5 μ in diameter, usually late in appearing ; chlamydospores, round or oval, rather thick-walled, hyaline, sometimes in short chains, 5 to 10 μ in diameter.

Parasitic in roots of Cajanus indicus and saprophytic in soil, India.

Other biological characters of Fusarium udum.

The fungus remains alive in soils, even in the absence of the host plant, for a considerable period. The pot experiments have

not been of sufficient duration to indicate how long this period may be, but it certainly exceeds a year. Most of the conidia found in cultures nearly a year old were still capable of germination, and then usually formed secondary spores very rapidly. The chlamydospores are likely to retain their vitality for a still longer period. The prevalence of the disease and the extent of damage caused by it are largely due to soil infection from the preceding crop. It is on this account that sufficient rotation is of such paramount importance in pigeon-pea cultivation in India. Spread within a field takes place chiefly through the soil. It is, however, dependent largely on the space between the plants and the size of the latter. It is noticeable that extension occurs much more rapidly where the plants are crowded in pots than in the field. Similarly the extension is more rapid the larger the plants are. It cannot be doubted that this is due in both cases to extension occurring more rapidly along the roots than across the intervening soil. Hence the nearer together the roots of different plants are, whether as a result of overcrowding or of extensive lateral growth in the roots of large plants, the more rapid the spread of the disease.

Aerial spread by wind-borne spores probably occurs, though to a lesser extent and much more slowly than through soil. The following observations bear on this point. Up to the present no direct attempt to isolate the parasite from the bark of the stem of wilted plants has been made, though it is proposed to do so shortly. Fusarium beds and microconidial colonies are common on such places, but they have not been separated and their parasitism tested. Some at least belong to other species. The control pots growing in the neighbourhood of infected pots have in several cases ultimately developed the disease. This has not occurred until the plants were a good size, from three to five months after sowing. The control plot of Series XVI remained free from wilt for eight months, though only separated from the inoculated plot by a narrow trench a foot deep. Aerial infection is not likely to be responsible for much loss since it occurs rather late in the season, and though isolated plants may be killed before harvest, centrifugal spread in

the soil to surrounding plants has not time to occur to any great extent.

There is no definite evidence that the disease is conveyed in the seed, and this appears to be unlikely. The control plants in the earlier series, before seed disinfection was practised, indicate that it does not happen commonly.

To determine the exact locality of infection a series of water cultures was tried, the plants being germinated under sterile conditions, and transferred to plugged sterile cylinders containing von Crone's solution,[1] when the shoot was from $1\frac{1}{2}$ to 2 inches high. Arrangements were made for daily aeration of the solution and stirring up the precipitate of salts. The inoculations were made from a glucose agar culture. The results bore out, what was already fairly evident from examination of the pot cultures, that infection begins ordinarily through the fine lateral roots. These were rapidly attacked and shrivelled, whether they arose below the seed, or above it, in those cases where the seed was well below the cork. On the other hand, inoculation of the stem gave no result, nor did one or two inoculations made on the main root just below the seed and above the origin of the first lateral root. The water cultures were remarkable in some respects. Though infection occurred on the white lateral roots, even more than an inch behind the growing point, provided the part inoculated was above the level of the solution, none occurred in any case below the liquid. The filaments in the former case penetrated the cortex and led to browning of the cortical cells, visible externally as a distinct brown mark at the point of inoculation. They did not, however, reach the vascular system, no case of penetration of the central cylinder having been observed. Hence there was no stoppage of the upward current and death did not result in any of the six plants inoculated at the roots, though two of them shed many of their leaves and looked very pallid, and a third was less markedly affected. In two of these the observations extended for over three months, in the other four for two

[1] Potassium nitrate 1 grm., ferrous phosphate ·25 grm., tri-calcic phosphate ·25 grm., magnesium sulphate ·5 grm., calcium sulphate · grm., water 1 litre.

months. Schikorra [(14) p. 16] similarly found that the parasitism of the "St. Johannis" disease parasite in water cultures was less than in soil. In inoculations below the liquid the explanation may simply be that the destructive enzymes by means of which penetration is effected are diluted as soon as formed and never reach a concentration sufficient to injure the roots. This does not explain, however, why the vascular system was not penetrated in the cases inoculated above the water level, and the problem involved is evidently of great physiological interest.

Treatment.

In the case of an internal root parasite widely distributed in the ordinary cultivated soils of the parts of India where pigeon-pea is grown, nothing short of extensive sterilisation of the soil will free it from the germs of the disease, if frequently cropped with the host plant. Such sterilisation is usually not practicable on account of the high cost, and is besides very difficult in the wilts hitherto investigated. Orton (3) tested the effects of a large number of soil fungicides in the allied cotton wilt, and failed to obtain satisfactory results with any. Delacroix (5) advocates the use of formaline injected into the soil, but the cost of his treatment, which he claims was perfectly efficacious against carnation wilt, works out to nearly an anna per square yard, or Rs. 300 per acre. I have tried sulphate of iron and also lime, with results agreeing with those of Orton. The sulphate of iron was applied directly to the soil around each plant, half a pint of a 2½ per cent. solution to each of 323 plants. No benefit whatever resulted. The lime was applied at the rate of one ton to the acre, the plots being 400 square feet in area. In the two limed plots 70 and 45 plants respectively had wilted at the end of 5 months, quite as many as in the similar unlimed plots near by. Bolley (19) working with the flax wilt Fusarium found it was rather more virulent in limed than in ordinary soils.

No application made to the above-ground parts of the plant can possibly do any good, since the fungus lives in the soil and attacks the plants through the roots.

There remains only, in our present knowledge of plant therapeutics, the method of breeding resistant strains. This method has already proved extremely fruitful in the United States. In India the introduction of *buri* cotton into wilt affected parts of the Central Provinces promises equally well. In any case it is the only method at present available and must therefore be fully tried.

Preliminary work was commenced in 1906, under the care of Mr. J. B. Knight, Professor of Agriculture, Bombay Department of Agriculture. The number of varieties of pigeon-pea grown in India does not appear to be large. All those that could be found were collected and sown on the Poona Farm in 1905. In 1906, nine of the varieties that promised best were resown on the same plot. The following year four varieties were retained. The purpose of continuing the growth of certain varieties on the same plot, prior to single plant selection, was twofold. It was desired to learn something of their value, and it was necessary before definite selection work was commenced, to make sure that all the plants had been exposed to the disease.

In 1908, the control of the selection work was taken over by Mr. W. Burns, Economic Botanist to the Government of Bombay, who is continuing it on the lines of pedigree breeding from single plants.

The most recent work in plant improvement has shown that individual plants which possess a given character, frequently transmit this character unaltered to their progeny. Individual plants differ greatly in regard to this ability, and these differences may apply to a whole variety (*e. g.*, the Egyptian varieties of cotton are generally more resistant to the American cotton wilt, than the indigenous Upland varieties, and the same seems to apply to *buri* cotton in regard to the cotton wilt in India). No variety of pigeon-pea obtained has shown itself to be specially resistant to the disease. More usually the inherent resistance is confined to individual plants and the behaviour of the other plants in the variety need not be considered.

Pedigree breeding consists simply in separating out once for all the individual plants possessing the required character, and multiplying rapidly, under isolation, their progeny.

The first step in pedigree breeding for disease resistance is to choose a considerable number of plants which are both resistant to the disease, and possess otherwise desirable qualities, such as productiveness, etc. This is simple enough in the case of a disease which spreads rapidly by air-borne spores, since in any year of considerable disease the individual plants in a plot will be all equally exposed to infection. In a disease of the type of wilt, where spread occurs comparatively slowly through the soil, it is not possible to be sure, in an experiment lasting through one season only, that healthy plants are resistant to the disease or have merely escaped infection through not coming into contact with the parasite. Hence in the experiments at Poona the seed of selected healthy plants was resown on the same plot for several seasons, until the whole of the soil was thoroughly infested and the possibility of any plant not being reached by the parasite was reduced to a minimum. It was early apparent that there was little hope of any *variety* proving resistant, as the plots became a mass of mixed types, presumably through free intercrossing. In the last season or two the crossing, if effected between plants within the plot, has been probably in most cases between more or less resistant individuals, and the individual plants possessing the quality of resistance inherent in them are, it is hoped, now fairly numerous.

The seed of the selected plants will be harvested separately and planted separately next season. The resulting growth and behaviour in regard to the disease will be critically examined. If the desired character of resistance appears definitely in any of the plots, combined with other desirable characters sufficiently to make the variety suitable for general cultivation, it will be rapidly multiplied and tested on a large scale for distribution. Should the first selections not prove successful, they must be repeated. Ultimately, though experience is as yet comparatively limited with

regard to this method of combating disease, it may be hoped to obtain a variety that is truly resistant to wilt.

It is only in this direction, so far as our knowledge at present extends, that a practical means of fighting the disease can be sought.

PUSA, *March 30th*, 1909.

DESCRIPTION OF THE PLATES.

PLATE I. *Neocosmospora vasinfecta Smith, from indigo (Indigofera sumatrana).*

Fig. 1. Perithecia on a root. × 4.

„ 2. Pure culture on nutrient agar from an ascosporal colony, showing concentric formation of perithecia in culture. × 4.

„ 3. A perithecium from pure culture on nutrient agar. × 170.

„ 4. Ascospores from the same culture as fig. 3. The projections of the outer wall are shown too regular. The right hand spore is focussed in surface view to show the superficial markings. × 1100.

„ 5. Asci with spores, and paraphyses, from the same culture as above. ×580.

(*All painted by H. Hormusji*).

PLATE II. *Neocosmospora vasinfecta Smith, from ascosporal cultures.*

Fig. 1. Fusarium spores (macroconidia). Indigo fungus. × 300.

„ 2. Germination of ditto, with secondary formation of microconidia from the upper spore and of a macroconidium from the lower spore. Pigeon-pea fungus. × 300.

3. Intermediate types between micro- and macroconidia. Indigo fungus. × 300.

„ 4. Chlamydospores. Indigo fungus. × 300.

„ 5. Formation of microconidia. From a continuous observation. Pigeon-pea fungus. × 325.

„ 6. Mycelium bearing microconidia formed from germination of the ascospores. Cotton fungus.

„ 7, 8 & 9. Perithecia and ascospores of the gram, pigeon-pea and cotton fungus respectively. Perithecia × 114, spores × 730.

PLATE III.

Fig. 1. Fusarium udum n. sp., pure culture on alkaline potato (see text). × 1½.

„ 2. Ditto on acid potato. × 1½.

„ 3. Ditto on acid rice. Natural size.

„ 4. Neocosmospora vasinfecta Smith, pure culture on acid rice. Natural size.

„ 5. Ditto on bread. Natural size.

„ 6. Germination of the ascospores of Neocosmospora vasinfecta from cotton.

(*All painted by A. N. Banerji*).

PLATE IV. *Fusarium udum n. sp.*

Fig. 1. Whorled conidiophore with microconidia. × 730.

„ 2. Simple lateral conidiophores on the creeping mycelium, with microconidia. × 730.

Fig. 3. Aerial conidiophore with microconidia in heads (Cephalosporium type). × 730.

„ 4. Types of microconidia from a single culture on nutrient agar 3 days old × 730.

„ 5. Formation of microconidia. From a continuous observation. × 730.

„ 6. Germination of the microconidia. × 730.

„ 7. Fusarium stage (macroconidia) with mode of attachment. The lower four spores were formed from the microconidia figured in fig. 6. × 730.

„ 8. Chlamydospores. × 730.

„ 9. Germination of the macroconidia with formation of secondary spores. From an old maltose culture. × 330.

„ 10. Anastomosis between the germ tubes of two macroconidia. × 330.

PLATE V.

Fig. 1. Early stage of root infection in pigeon-pea wilt. The seedling was sown in infective soil on November 21st, 1907, and was removed on March 28th, 1908. Eight seedlings had wilted between January and March. × $1\frac{1}{4}$.

„ 2. Stages in pigeon-pea wilt. From a photograph.

PLATE VI.

Fig. 1. Two of the pots of Series XV. One inoculated with Fusarium udum n. sp. and one not inoculated (see text). From a photograph.

„ 2. Three of the pots of Series XIV, two inoculated with Fusarium udum n. sp. and one not inoculated (see text). From a photograph.

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PLATE I.

2

1

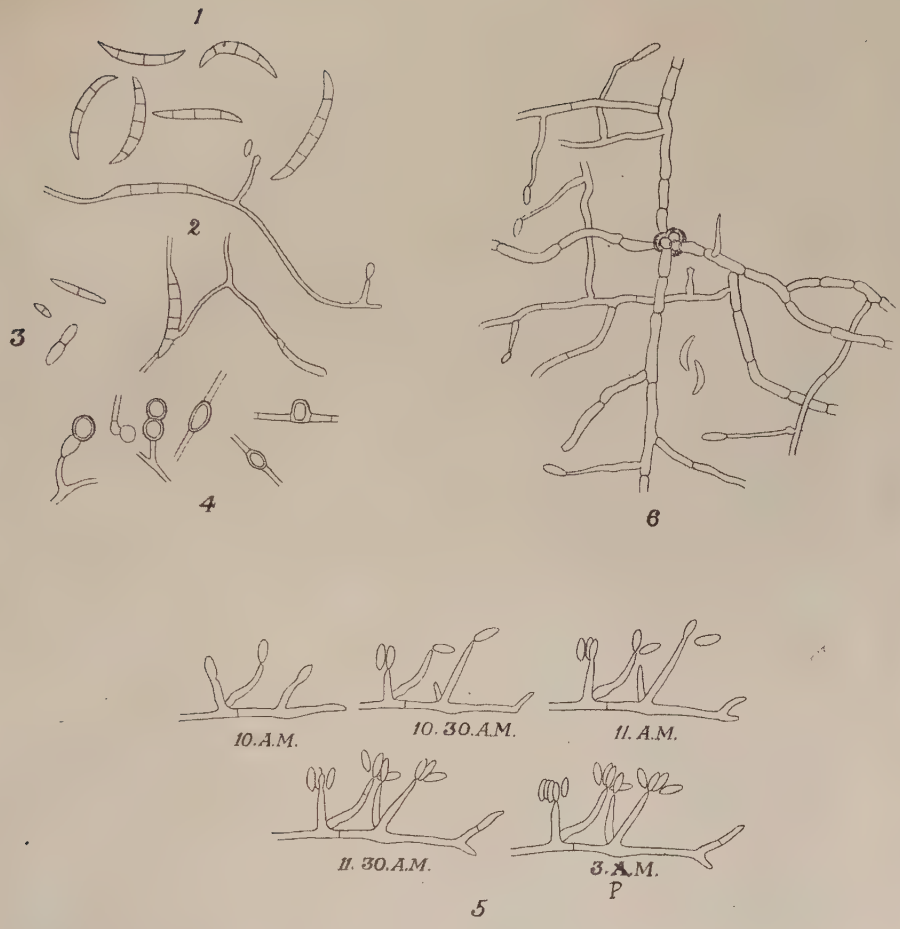
4

3

5

NEOCOSMOSPORA VASINFECTA.

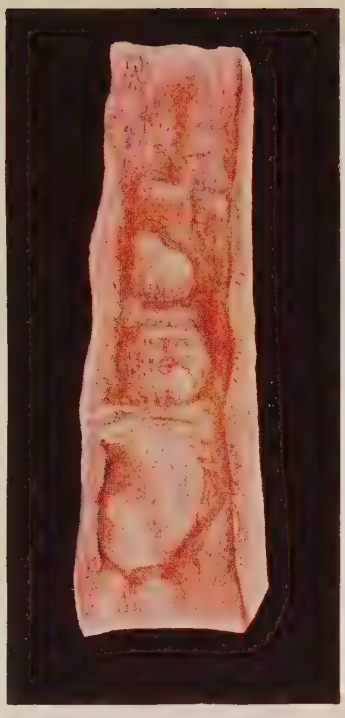
PLATE II.



NEOCOSMOSPORA VASINFECTA.

PLATE. III.

1



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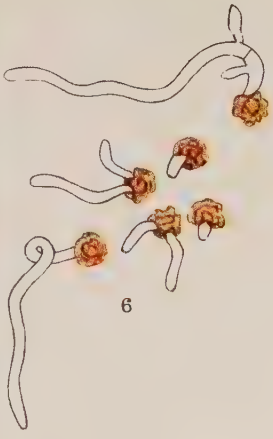

6

FUSARIUM UDUM (FIGS. I TO 3) AND
NEOCOSMOSPORA VASINFECTA. (FIGS. 4 TO 6).

Engraved and Printed by THE CALCUTTA PHOTOTYPE

PLATE V.

Fig. 1.

Fig. 2.

PIGEON-PEA WILT.

PLATE VI.

Fig. 1.

Fig. 2.

PIGEON-PEA WILT.

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